Posters

Adolescent & Young Adult Sarcomas

(P 01) EXTRASKELETAL EWING SARCOMA OF THE EXTREMITIES AND TRUNK IN < 25 YEARS OLD PATIENTS: A RETROSPECTIVE ANALYSIS OF A MONO-INSTITUTIONAL SERIES.

Giuseppe Bianchi, MD[1]; Roberta Laranga, PhD[1]; Maria Antonella Laginestra, PhD[2]; Laura Pazzaglia, PhD[2]; Fabiana Macri, n/a[3]; Federico Ostetto, Dr.[4]; Anna Paioli, MD.[5]; Toni Ibrahim, BSc, MSc, MD, PhD[5]; Marco Gambarotti, MD[6]; Katia Scotlandi, PhD[2]; Davide Maria M. Donati, MD[7], (1) Istituto Ortopedico Rizzoli IOR IRCCS, SC Third Orthopaedic Clinic and Traumatology, Bologna Italy, BOLOGNA, Emilia-Romagna, Italy, (2) Istituto Ortopedico Rizzoli IOR IRCCS, Laboratory of Experimental Oncology, Bologna, Italy., Bologna, Emilia-Romagna, Italy, (3) UNIBO, Bologna, Emilia-Romagna, Italy, (4) Istituto Ortopedico Rizzoli IRCCS, Bologna, Emilia-Romagna, Italy, (5) Istituto Ortopedico Rizzoli IRCCS, Osteoncology- Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (6) Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (7) Istituto Ortopedico Rizzoli IOR IRCCS, SC Third Orthopaedic Clinic and Traumatology, Bologna Italy, Bologna, Emilia-Romagna, Italy

Objective: Extra skeletal Ewing's sarcoma (EES) is a malignant tumour belonging to the undifferentiated round cell sarcoma tumours (USRCS). Although current international recommendations indicate that EES should be treated with the same principles as for bone Ewing Sarcoma (Bone-ES), the optimal treatment strategy of EES remains uncertain. We have retrospectively analysed the clinical features, treatments, and outcomes of patients aged minor of 25 years with EES treated in our centre. Moreover, we compared the genetic profile of EES and Bone-ES to highlight differences between these entities.

Methods: Nineteen EES patients (12-25 years), treated at the Istituto Ortopedico Rizzoli of Bologna (Italy), from 1988 to 2022, were identified. Diagnosis was reviewed according to the WHO classification 2020. All cases were evaluated for specific chromosomal translocations involving EWSR1 or FUS. The extra-skeletal origin was determined on radiological imagining. Patients with visceral or head-neck primary location were excluded. Approval by the Local Ethical Committee was obtained. The clinic-pathological variables analyzed include: sex, age, location, depth, size, synchronous metastasis, baseline serum lactate dehydrogenase, tumor's initial presentation (primary, unplanned/recurrence), local treatment, surgical margins, systemic treatment, histopathological response after chemotherapy (according to the Bologna System). Overall survival (OS), and Event-free survival (EFS) were estimated by Kaplan-Meier. Univariate (log rank test) and multivariate analysis (cox proportional hazard model) were done to explore prognostic factors. We performed RNA sequencing in 30 Small Round Cell Sarcomas (SRCSs) including, 18 Ewing (12 Bone-ES and 6 EES), 9 CIC-DUX4-rearranged sarcomas (CDS), 3 BCOR::CCNB3 sarcoma (BCS). Paired-end libraries were synthetized using TruSeq RNA Library Prep Kit for Enrichment and loaded on NovaSeq 6000 platform (Illumina), and were aligned to the GRCh38 human genome by STAR algorithm. FeatureCounts tool and DEseq2 were used to assign aligned reads to genes and to perform normalization applying variance stabilization transformation (vst). Uniform Manifold Approximation and

Projection (UMAP) for non-linear dimensionality reduction was employed for visualizing sarcoma groups in two dimensions based on 8000 genes with highest variance.

Results: Sixteen patients (84%) presented as a primary tumor, 3 (16%) a recurrent disease and 2 (10%) as “unplanned excision”. Five patients (26%) were metastatic at diagnosis. The cumulative 5-year OS rate was 67% (95%CI 39-83) (Figure 1). Size (HR=9 P=0.019) and tumor’s initial presentation (HR=12 p=0.013), resulted statistically correlated to OS (Figure 2A-B). Local recurrence occurred in 1 patient (5%), at 4 months from diagnosis; distant metastases occurred in 6 patients (32%), at a mean time of 21.5 months (95% CI 5-38): 4 cases in the lung (66%), 1 in lung and abdomen (17%), 1 in the bone (17%). The 5-year EFS rates was 52% (95% CI 28-72) (Figure 3A). Statistical evaluation revealed tumor’s initial presentation and size to be independent risk factors associated to EFS (HR=16 p=0.003 and HR=6 p=0.033, respectively) (Figure 3B-C). Histologic response to neoadjuvant chemotherapy (Ewing-like protocol) was analyzed with the Bologna System score. Overall, GR had a better survival (100% vs 50% of PR, p=0.1397). By dimensionality reduction using UMAP, the four SCRSs subtypes showed a clear separation, we did not observe differences in the transcriptional profile between EWSs arising in bone compared to soft tissue EWSs (Figure 4).

Conclusion: Recurrent/unplanned tumors and big dimension resulted to be independent prognostic factor related to OS and EFS. Based on the genes with highest variance, unsupervised analysis highlighted a similar transcriptional profile in EWS Sarcoma beside tumor’s tissue origin. This result supports the use of similar therapeutic approach among EWSs sarcomas.

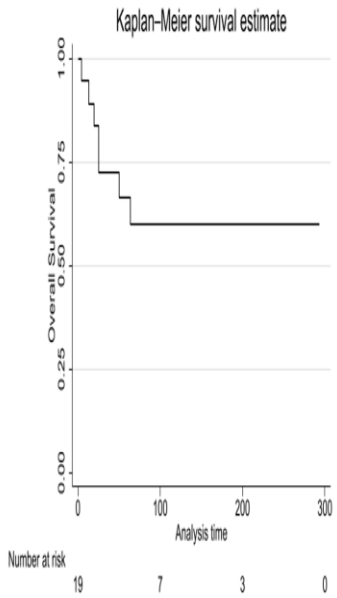


Figure 1: Overall survival estimation.

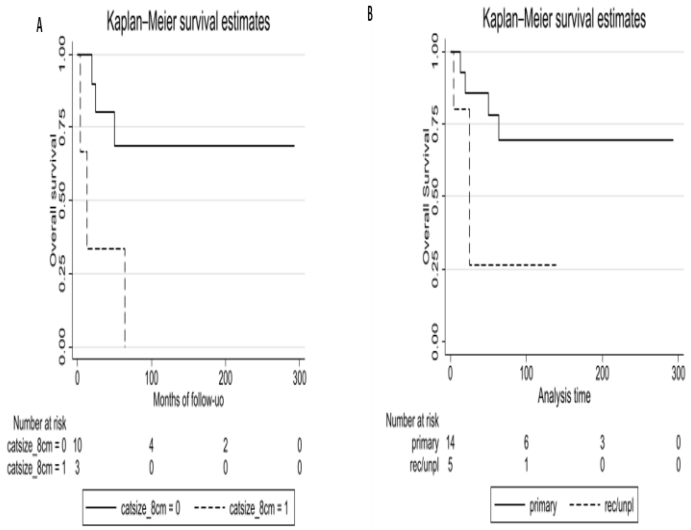


Figure 2: Overall survival estimation. A) Overall survival stratified by tumors' size (catsize_8cm=0 ≤8cm; catsize_8cm=1 >8 cm); B) Overall survival stratified by tumor's initial presentation (primary= primary tumor at presentation; rec/unpl: recurrent or unplanned tumor at presentation).

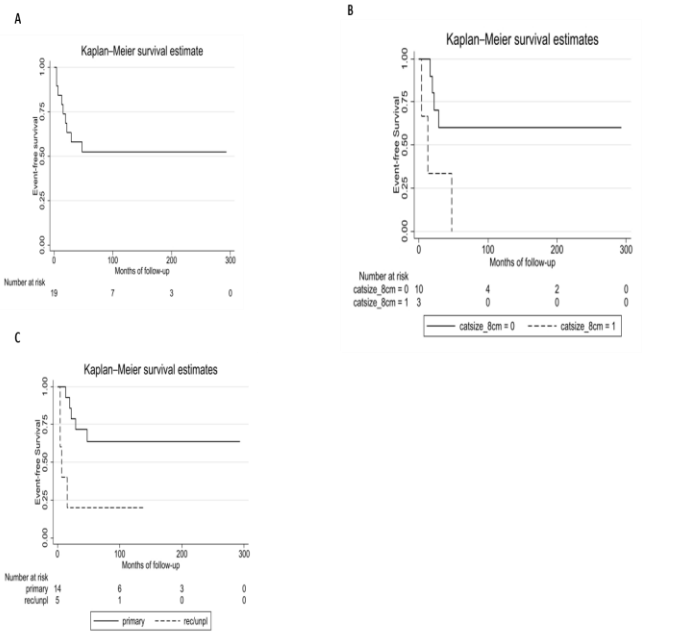


Figure 3: Event-free Survival estimation. A) Event-free survival (EFS); B) EFS stratified by tumors' size (catsize_8cm=0 ≤8cm; catsize_8cm=1 >8 cm); C)EFS stratified by tumor's initial presentation (primary= primary tumor at presentation; rec/unpl: recurrent or unplanned tumor at presentation).

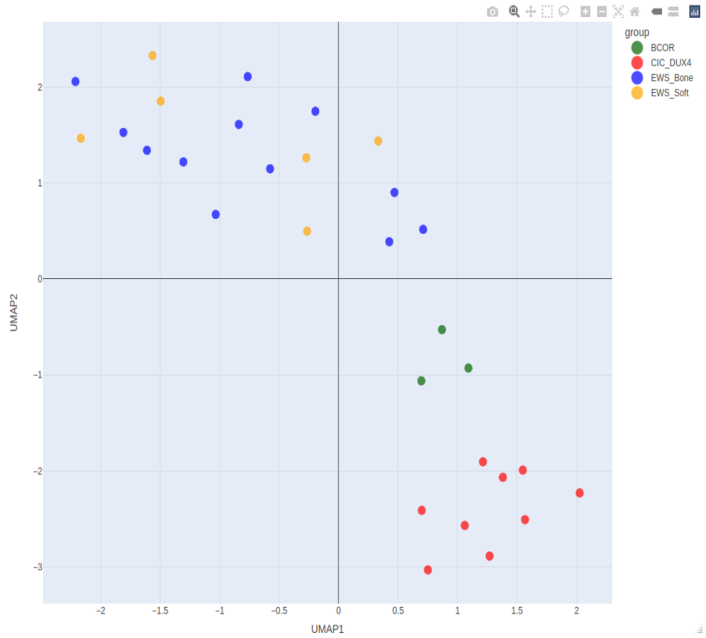


Figure 4: Uniform Manifold Approximation and Projection (UMAP) for non-linear dimensionality reduction

(P 02) PEDIATRIC SYNOVIAL SARCOMA: A REVIEW OF 21 CASES FROM A SINGLE INSTITUTION

Samuel Broida, N/A, MD[1]; Mikaela Sullivan, MD[2]; Alexandra Arguello, MD[2]; Steven Robinson, MBBS[2]; Scott Okuno, M.D.[2]; Brittany Siontis, MD[2]; Thanh P. Ho, MD[2]; Ivy A. Petersen, MD[2]; Safia K. Ahmed, MD[3]; Meng Xu-Welliver, MD, PhD[2]; Wendy A. Allen-Rhoades, N/A, MD[2]; Matthew T. Houdek, MD[2], (1) Mayo Clinic (Rochester), Rochester, MN, (2) Mayo Clinic, Rochester, MN, (3) Mayo Clinic Arizona, Phoenix, AZ

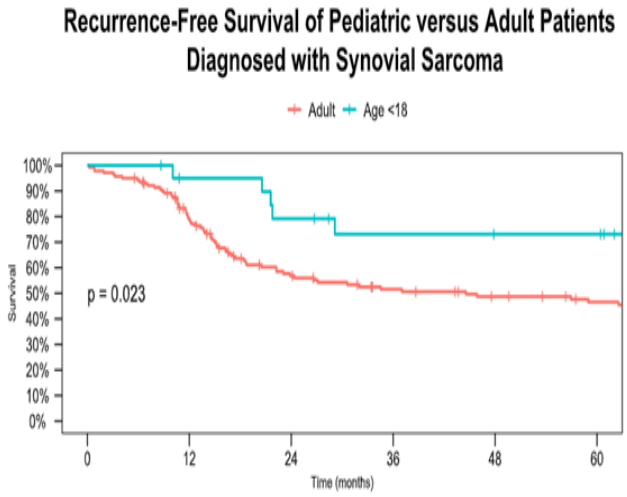
Objective: Synovial sarcoma is rare, yet remains the most commonly diagnosed soft tissue sarcoma in childhood and adolescent patients. There are few studies reporting on the outcomes of synovial sarcoma in patients under 18 years of age. We sought to describe the rates of recurrence and disease-specific survival in pediatric patients diagnosed with synovial sarcoma.

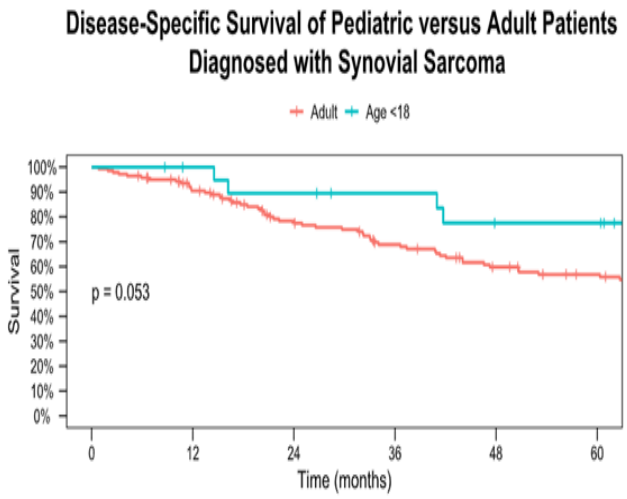
Methods: We retrospectively reviewed the medical records of 361 patients with synovial sarcoma. Twenty-one patients (6%) were diagnosed with synovial sarcoma prior to the age of 18. All patients were evaluated at a single institution between 2000 and 2020. Mean age at diagnosis was 11 years (range 4 – 17) and 13 patients (62%) were male. Nineteen primary lesions were located in the extremity, one tumor was located in submandibular region, and one tumor was located in the buttock. The mean maximum pre-treatment dimension of primary lesions was 4.7±2.8cm (range 1.1-10.8). Seven patients were diagnosed via contaminated excisional biopsy. Fifty-two percent of tumors were of the monophasic subtype and the remainder were biphasic. Median follow-up for survivng patients was 62 months (range 9-192).

Results: All patients underwent surgical treatment of the primary lesion in the form of limb salvage. Seven patients with low risk lesions (< 5cm, completely resected) were treated with

surgery alone. Eleven patients received both perioperative chemotherapy (doxorubicin/ifosfamide [n=9], VDC/IE [n=2]) and radiotherapy. Three patients received perioperative radiotherapy (mean 50Gy) without chemotherapy. Two patients had positive margins, one of which was a planned intralesional procedure and was managed via brachytherapy and the other was an unplanned positive margin that was addressed with post-operative radiation.
Eleven patients (52%) underwent reoperation for infection/wound complications (n=5), scar tissue (n=2), recurrence (n=2), leg length discrepancy following rotationplasty (n=1), and amputation for radiation-associated osteosarcoma (n=1). Three patients developed local recurrence at 5, 20, and 21 months. Only one of these patients had positive margins. Four patients developed lung metastases at a mean 20 months from biopsy. All four patients who developed metastases ultimately succumbed to disease. Mean survival from metastatic diagnosis was 11 months (range 4-21). Recurrence-free survival (RFS) at 1 and 5 years was 95% and 73%, respectively. Disease-specific survival (DSS) was 100% at 1 year and 78% at 5 years. Compared with adult patients with synovial sarcoma, pediatric patients had a lower risk of recurrence (HR 0.36, 95% CI [0.15, 0.90], p=0.02) and higher disease-specific survival (HR 0.38, 95% CI [0.14, 1.05], p=0.05) (Figures 1 &2)

Conclusion: Pediatric patients comprise a small percentage of patients diagnosed with synovial sarcoma. Local control including limb salvage surgery and perioperative radiotherapy resulted in low rates of local recurrence, but high rates of reoperation. Compared with adult patients, pediatric patients have higher recurrence-free and disease-specific survival rates. Overall, surgical treatment of pediatric synovial sarcoma with neoadjuvant or adjuvant chemoradiation, when indicated, results in high 5-year survival for the majority of patients.





(P 03) EVOLUTION OF CLINICALLY ACTIONABLE VARIANTS IN ADVANCED PEDIATRIC AND AYA BONE SARCOMAS

Hannah Y. Comeau, MS[1]; Lorena Lazo de la Vega, PhD[2]; Luke D. Maese, DO[3]; Navin Pinto, MD[4]; Margaret E. Macy, MD[5]; Mark Applebaum, MD[6]; Amit Sabnis, MD[7]; Steven G. Dubois, MD, MS[8]; Alanna J. Church, MD[9]; Katherine A. Janeway, MD[9], (1) Dana-Farber / Boston Children's Cancer and Blood Disorders Center, Boston, MA, (2) Dana-Farber Cancer Institute, Boston, MA, (3) Huntsman Cancer Institute/University of Utah Health, Salt Lake City, UT, (4) Seattle Children's Hospital, Seattle, WA, (5) Children's Hospital Colorado, University of Colorado School of Medicine, Aurora, CO, (6) Comer Children's Hospital/University of Chicago, Chicago, IL, (7) UCSF, San Francisco, CA, (8) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA, (9) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA

Objective: Few pediatric and AYA sarcoma studies have investigated how gene alterations detected by targeted cancer panels change in the same patient across multiple tumor samples over time. Our study leverages targeted somatic DNA next-generation sequencing (NGS) data from a large cohort of young patients with osteosarcoma (OS) or Ewing sarcoma (EWS) to identify changes in oncogenic variants and variants potentially predicting response to matched molecularly targeted therapy (MTT) with the aim of addressing the clinical utility of biopsy and resequencing pediatric and AYA bone sarcomas at recurrence.

Methods: The iCat2/GAIN (Genomic Assessment Informs Novel Therapy) Consortium Study is a multicenter cohort study involving 13 US cancer institutions evaluating outcomes after receipt of MTT in young patients with advanced extra-cranial solid tumors. Tumors are sequenced using OncoPanel, a targeted DNA NGS test performed in a CLIA/CAP certified laboratory assessing single nucleotide variants (SNV), copy number variants (CNV) and structural variants

(SV) in up to 447 cancer genes and rearrangements in up to 60 cancer genes. In 1 case, a similar targeted NGS tests was performed. Multiple tumor samples from the same patient at different timepoints (initial diagnosis or relapse) and tumor sites (primary or metastatic) are sequenced when available.

We selected OS or EWS patients with at least 2 successful targeted NGS panels. We assessed changes in pathogenic variants in genes that are either: 1) known to be biologically important in OS or EWS (oncogenic variants), or 2) gene alterations which may predict response to matched MTT (actionable variants). We categorized patterns of change as emergence, disappearance, or persistence. Pattern of change was variant-based rather than patient-based, so an individual patient could have more than one pattern present.

Results: Of 784 iCat2/GAIN patients there were 155 patients with OS and 107 with EWS. The analytic cohort consisted of 27 OS patients and 19 EWS patients with at least 2 tumor sequenced samples. The median number of sequenced samples per patient was 2 (range 2 to 6).

In EWS, 10 (53%) patients had an oncogenic or actionable variant in at least one sample. The predominant pattern of change in EWS was emergence of oncogenic and actionable variants occurring in 9/10 patients. Of the 19 EWS patients with >2 samples, 26% had emergence of a new pathogenic SNV in STAG2 or TP53 and 21% had emergence of new actionable variants in other genes (in MAP2K, FLCN, FGFR1, MYC, and CDKN2A). Persistence of oncogenic and actionable variants was also seen in 26% of patients. Disappearance of oncogenic or actionable variants was only seen in one case (in CCND1).

In OS, all 27 patients had at least one oncogenic or actionable variant in at least one sample. The predominant pattern of change in OS was persistence of oncogenic or actionable variants occurring in 22/27 patients, most commonly in TP53 (n=17), CDK4 (n=4), RB1 (n=4), CCND3 (n=3), and MCL1 (n=3). Emergence of new oncogenic or actionable variants occurred in 14/27 (52%) patients, with emergence of new TP53 mutations being the most common (n=8). There was also a high rate of disappearance of variants [12/27 (44%) patients], with disappearance of MYC amplification (n=6) and TP53 mutations (n=6) being the most common.

Conclusion: In EWS we observed a predominant pattern of emergence of new biological and/or actionable variants over time with emergence of new STAG2 or TP53 mutations occurring in 26% of patients, confirming the importance of these genes in resistant disease. In OS, we observed mixed patterns of change including persistence, emergence and disappearance of variants, suggesting the previously described unstable genome in OS impacts key oncogenes and potentially targetable genes. Given the observed patterns of genomic change in OS and EWS, there may be utility to repeat sequencing of relapsed tumor biopsies.

GAIN ID	Stage at DX	STAG2 DX	STAG2 R	TP53 DX	TP53 R
147	Localized		p.N641*		
278	Localized			p.R273C	p.R273C
29	Loco-regional				p.K139N
157	Distant metastasis	splice site	splice site		p.R213*
498	Distant metastasis		p.Y636*		
599	Distant metastasis	p.R216*	p.R216*		p.R249S

Table 1. Evolution of STAG2 and/or TP53 variants in Ewing sarcoma patients at relapse.

(P 04) **THE CELL ATLAS OF CLEAR CELL SARCOMA**

Christopher Davies, MSci[1]; Fernanda Amary, MD-PhD[2]; Adam Cribbs, PhD[3]; Jonas Demeulemeester, PhD[4]; Akanksha Farswan, PhD[5]; Adrienne Margaret Flanagan, MD-PhD[1]; Muhan Ju, BSc[6]; Daniel Lindsay, MD[7]; Jolet Y. Mimpen, DPhil[3]; Lorenzo Ramos-Mucci, PhD[3]; Nischalan Pillay, MBChB, FRCPath, PhD[1]; Jana Siebenlist, BSc[8]; Sarah Snelling, PhD[9]; Sandra J. Strauss, MD PhD FRCP[10]; Roberto Tirabosco, MD[2], (1) University College London, Cancer Institute, London, England, United Kingdom, (2) Department of Histopathology, Royal National Orthopaedic Hospital, Stanmore, UK, London, England, United Kingdom, (3) University of Oxford, Oxford, England, United Kingdom, (4) VIB-KU Leuven Center for Cancer Biology, KU Leuven, Vlaams-Brabant, Belgium, (5) University College London Cancer Institute, London, England, United Kingdom, (6) UCL, London, England, United Kingdom, (7) Royal National Orthopaedic Hospital NHS Trust, London, England, United Kingdom, (8) University of Heidelberg, Heidelberg, Baden-Wurttemberg, Germany, (9) University of Oxford, Oxfordshire, England, United Kingdom, (10) UCL Cancer Institute, London, England, United Kingdom

Objective: We hypothesize that combined sequencing of single nuclei extracted from a well characterised cohort of clear cell sarcomas combined with bulk DNA, RNA and methylation analysis will help identify the normal cell components along with subpopulations of tumour cells. This information could open the opportunity to identification of therapeutic vulnerabilities.

Methods: Fresh frozen samples of clear cell sarcoma were sourced from the Royal National Orthopaedic Hospital NHS trust, London, UK and processed for multi-omic analysis. Nuclei were extracted from frozen tissue using a Tris Tween based dissociation and run through the 3 prime 10X genomics RNA sequencing pipeline. 5 primary cases and 5 metastatic cases were sequenced, with 2 cases having matched primary and metastatic regions. Using the R package Seurat, data was quality controlled, clustered, and annotated based on their RNA signatures.

These clusters were then compared with other sequencing and histological data to produce a robust set of identities for the cells annotated in the data. Data for other tumour cell types were accessed via the GEO repository and a cohort of normal cell types arising from the tendon were made available by the Human Tissue atlas group based in Oxford, UK. This research was supported by the generous philanthropic gift to UCL by the Edward Showler Foundation. https://www.edwardshowlerfoundation.com/

Results: Cell annotations confirm the neural crest and melanocytic program that is active in the tumour cells and associates with expression of SOX10, MITF and PMEL. Clustering revealed a minimum of three distinct populations of tumour cells in each sample which are underpinned by proliferation and cell differentiation trajectories. These correlate closely with the clonal and sub-clonal copy number gains seen in the whole genome sequencing. The immune contingent of the tumour is very low and predominantly comprised of macrophages with small clusters of T-cells.

Conclusion: Initial findings from our data look promising and have revealed novel insights into genomic and transcriptional heterogeneity in CCS. Analysis of longitudinal data and comprehensive characterisation of the differences between primary and metastatic tumours could boost our understanding of the evolution patterns in this cancer. The immune-excluded nature of the tumour suggests that conventional immune-checkpoint therapy on its own may not be a rational approach and that combination therapies or other forms of immune therapies such as CAR-T cell therapy may be worth pursuing.

(P 05) COEFFICIENT OF VARIATION OF T2-WEIGHTED MRI PREDICTS THE PROGNOSIS OF MALIGNANT PERIPHERAL NERVE SHEATH TUMOR

Makoto Emori, MD PhD[1]; Hiroyuki Tsuchie, MD[2]; Hiroyuki Takashima, PhD[3]; Yasutaka Murahashi, M.D.[1]; Junya Shimizu, M.D.[1]; Yoshinori Imura, MD, PhD[4]; Hidetatsu Outani, MD, PhD[4]; Sho Nakai, MD, PhD[5]; Satoshi Takenaka, MD, PhD[5]; Naoya Nakahashi, MD[1]; Hiroyuki Nagasawa, MD[6]; Seiji Okada, MD, PhD[4]; Naohisa Miyakoshi, MD, PhD[7]; Toshihiko Yamashita, President[1], (1) Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan, (2) Akita University Graduate School Of Medicine, Akita, Akita, Japan, (3) Hokkaido University, Sapporo, Hokkaido, Japan, (4) Osaka University, Osaka, Osaka, Japan, (5) Osaka International Cancer Institute, Osaka, Osaka, Japan, (6) Akita University Graduate School of Medicine, Akita, Akita, Japan, (7) Akita University Graduate School Of Medicine, Osaka, Osaka, Japan

Objective: Malignant peripheral nerve sheath tumors (MPNST) are rare and account for 2% of soft tissue sarcomas. To ameliorate clinical decision-making, it is important to establish several independent clinical factors associated with the survival of patients with MPNST. Therefore, we aimed to find an imaging-based prognostic factor of MPNST. An imaging-based predictor for the prognosis of MPNST has not been established. This study aimed to investigate whether non-enhancement MRI features, including measurement of the heterogeneity of the tumor with MR T2 imaging by calculating CV values, are associated with the prognosis of non-metastatic MPNST.

Methods: This retrospective study was conducted at four institutions. The inclusion criteria were as follows: histologically non-metastatic MPNST who underwent surgical resection. MRI was performed preoperatively. Non-enhancement MR images were evaluated for signal

intensity heterogeneity at T1/2-weighted imaging, tumor margin definition at T1/2-weighted imaging, peritumoral edema at T2-weight imaging, and CV. Two observers were blinded to the clinical information and analyzed the MR images. The following were recorded according to the previous reports : (a) tissue layer (deep or superficial); (b) longest diameter (in millimeters); (c) SI heterogeneity at T1/2-weighted imaging categorized as homogeneous, < 50% heterogeneous, or ≥ 50% heterogeneous (when 0%, < 50%, or ≥ 50% of tumor volume showed areas of low, intermediate, and high SI, respectively); (d) tumor margin definition (poorly defined [> 75% of margin not clear], mixed margin [10%- 25% of margin not clear], or well defined [> 90% of margin clear]); (e) peritumoral edema at T2-weighted imaging, defined as high fluidlike signal at T2-weighted or short tau inversion-recovery sequences imaging with infiltrative and feathery borders that are distinguishable from the apparent tumor boarders (defined by a pseudo capsule or non-equivocal changes in SI of other sequence) and without mass effects (defined as modification of the usual morphology of the local anatomy categorized as no, limited, or extensive when involving the entire length and breadth of the muscle).The tumoral region of interest (ROI) was measured, which showed the maximum tumor diameter. The ROI in the tumor was hand-drawn and set with its surrounding tumors (Fig. 1). We measured the signal intensities of MR T2-weighted images and calculated the corresponding CV values using Philips DICOM Viewer R3.0-SP15. CV is defined as the ratio of the standard deviation to the mean. The associations between factors and overall survival (OS) were investigated via Kaplan–Meier method with log-rank tests and the Cox proportional hazards model.

Results: From April 2002 to August 2022, 77 patients with MPNST were identified. We then excluded patients with metastatic lesions and unplanned excision at initial presentation and 13 patients without surgical treatment and MRI. Finally, 42 patients were included (Fig. 2). Patients comprised 20 men and 22 women. The mean patient age was 50 years (range, 8–93 years), and the mean patient follow-up period was 54.7 months (range, 2–217 months). The median tumor diameter was 82.5 mm (range, 26–250 mm). Demographic and clinicopathologic characteristics of the 42 patients are shown in Table 1. The 5- year OS rate for the entire patient cohort was 55.9%. The mean CV value of MR T2 images was 0.2299± 0.1339 (SD) (range, 0.0381–0.8053). Applying receiver operating characteristics analysis, the optimal cut-off level for CV value was 0.137. This cut-off CV value was used for its stratification into high and low CV values. The results of univariate survival analyses of prognostic factors for OS are listed in Table 2. At multivariate survival analysis, a high CV value (hazard ratio = 3.63; 95% confidence interval = 1.16–16.0; p = 0.047) was identified as an independent predictor of OS. Interobserver and intraobserver reproducibility for the calculation of CV value was 0.99 (95% CI = 0.97-1.00) and 0.99 (95% CI = 0.99-1.00), respectively.

Conclusion: We focused on the heterogeneity of MPNST to evaluate an imaging-based prognostic factor. CV was used to reflect tumor heterogeneity because MRI protocols were diverse. The prognosis of MPNST could be predicted based on CV values, which do not require an additional imaging modality and are quite reproducible.



Patients with primary malignant peripheral nerve sheath tumors (MPNSTs) (n=77)

- Patients with metastasis (n=7)
- Unplanned excision (n=15)

Patients with no metastatic and no unplanned excised primary MPNSTs (n=55)

- Patients without surgical treatment (n=4)
- MR imaging unavailable (n=9)

Included patients (n=42)

Variable	N (n=42)	%
Age		
<50	21	50
≥50	21	50
Gender		
Male	20	47.6
Female	22	52.4
NF 1 status		
Absent	24	57.1
Present	18	42.9
Location		
Extremity	15	35.7
Non-extremity	27	64.3
Grade		
Low (I)	8	19.0
High (II, III)	34	81.0
Size		
≤50mm	13	31
50～100	13	31
≥100mm	16	38
Depth		
Subcutaneous	12	28.6
Deep	30	71.4
Margin status		
R0	31	73.8
R1/2	11	26.2
Chemotherapy		
Yes	19	45.2
No	23	54.8
Radiotherapy		
Yes	13	30.1
No	29	69.1

Variable	N (n=42)	5-year Overall survival (%)	p value
Heterogenous SI at T1-weighted imaging			0.123
<50%	19	66.4	
≥50%	23	48.3	
Heterogenous SI at T2-weighted imaging			0.122
<50%	10	72.9	
≥50%	32	51.8	
Margin definition at T1-weighted imaging			0.036
Well	10	88.9	
Mix/Poor	32	46.0	
Margin definition at T2-weighted imaging			0.079
Well	8	87.7	
Mix/Poor	34	49.0	
Peritumoral edema			0.096
No	19	64.0	
Limited/Extensive	23	48.6	
Coefficient of variation on T2-weighted imaging			0.037
Low (<0.137)	13	82.5	
High (≥0.137)	29	43.1	

(P 06) ONCOFERTILITY AND PREGNANCY IN ADOLESCENT AND YOUNG ADULT CANCERS: PHYSICIANS' KNOWLEDGE AND PREFERENCES IN A LARGE LOW-MIDDLE-INCOME COUNTRY

Jyoti Bajpai, MD, DM[1]; Sharada Mailankody, MD, DM[2]; Puneet R Arora, MD[3]; Rinoy Sreedharan, MD[4]; Prakash Chitalkar, MD[5]; Purna Kurkure, MD[6]; Hemant Malhotra, MD[7]; Firuza R Parikh, MD[8]; Sudeep Gupta, MD, DM[9]; Shripad D Banavali, MD[10], (1) Tata Memorial Centre,Homibhabha National University,Mumbai,India, Mumbai, Maharashtra, India, (2) Department of Medical Oncology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India, Manipal, Karnataka, India, (3) Director, Center for Infertility and Assisted Reproduction (CIFAR), Gurugram, India, Gurugram, Delhi, India, (4) Indira IVF Bhandup, Mumbai, India, Mumbai, Maharashtra, India, (5) Sri Aurobindo Institute of Medical Sciences, Indore, India, Indore, Madhya Pradesh, India, (6) Narayana Health, SRCC Children Hospital, Mumbai, India, Mumbai, Maharashtra, India, (7) Sri Ram Cancer Center, Mahatma Gandhi Medical College Hospital, Jaipur, Rajasthan, India, (8) Jaslok hospital, Director Well Women Centre, HN reliance Hospital, Mumbai, Maharashtra, India, (9) Tata Memorial Centre, Mumbai, Mumbai, Maharashtra, India, (10) Tata memorial Centre, Mumbai, Mumbai, Maharashtra, India

Objective: Adolescent and young adult(AYA) cancers have prognostically improved with advanced oncology care and demand sustainable, precision care inclusive of fertility preservation(FP), including in low-middle income countries(LMIC).

Methods: An online survey link with 17 questions was circulated among oncologists on social media platforms and conference venues to assess the knowledge and seek suggestions to optimize referral pathways for FP.

Results: The median age of the 179 respondents was 37 (IQR:10,29-74) years, with a predominance of medical oncologists (68.7%), working in academic centers (39%) having median experience of 4(IQR 4,1-42)years; 23(12.8%) had dedicated AYA cancer units. Though a quarter (19-24%) of respondents discussed fertility issues in >90% of AYA patients with cancer, only a tenth (8-11%) refer>90% of these for FP, with significantly higher ($p < 0.05$) discussions and referrals in males and by more experienced oncologists ($p < 0.05$).
Forty-six (25.6%) were not well-versed with the international guidelines for FP. Most (122,68.1%) oncologists knew about the referral path for semen cryopreservation;however, only 46% were knowledgeable about additional complex procedures(testicular/ovarian cryopreservation, testicular sperm extraction). One-hundred and ten(61.5%) of oncologists never/or rarely altered the systemic treatment for FP.
Prominent barriers to FP were ignorance, lack of collaboration, and fear of delaying cancer treatment. Lead thrust areas identified to improve FP practices and outcomes are education, easy enhancement, and affordable access to FP facilities.
Seventy-four(41.3%) respondents knew about international guidelines for pregnancy with or after cancer; however, only half of them (20%) often monitored fertility outcomes in survivors. Oncologists have conflicting opinions and uncertainties regarding pregnancy safety, assisted reproductive techniques, breastfeeding, and oncologic and pregnancy outcomes among survivors.

Conclusion: There is uncertainty among oncologists regarding the guidelines, FP practices, referral pathways, and post-treatment pregnancies. A multi-pronged approach with focused AYA clinics with experts, improving awareness, and provision of easily accessible oncofertility

facilities is the need of the hour including in LMIC.

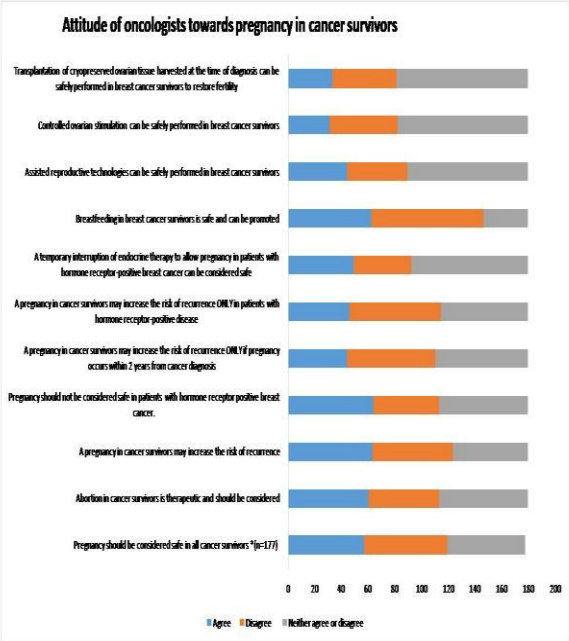


Figure 3. Attitudes of Oncologists towards pregnancy in cancer survivors

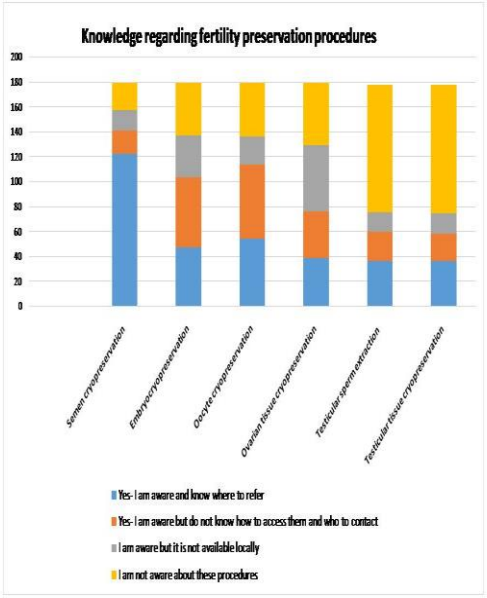


Figure 2. Level of awareness among oncologists regarding fertility preservation procedures

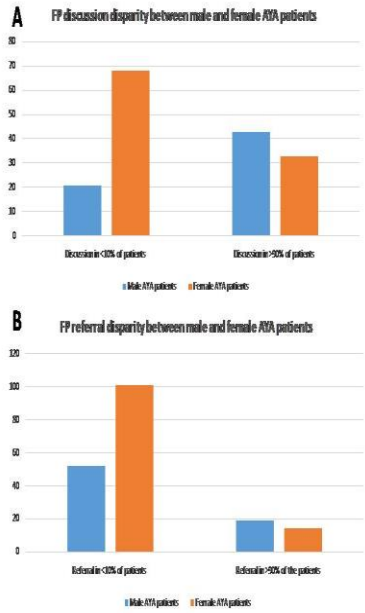


Figure 1. Gender differences in oncofertility practices. A: Differences in oncofertility discussions between male and women AYA patients. B: Differences in oncofertility referrals between male and women AYA patients.

AYA Adolescent and Young adult., FP fertility preservation

Table 3. Factors associated with oncofertility counselling (n=177)

Factor	No (%)	FP discussion *	p-value
Gender of respondent			
Female	53 (29.6)	8(15.1)	0.062
Male	124 (70.4)	35 (28.2)	
Age of respondent			
≤40 years	118 (66.5)	31 (26.2)	0.386
>40 years	59(33.5)	12(20.5)	
AYA special unit			
Yes	23(13)	3(13)	0.117
No	154 (87)	40(26)	
Years of clinical practise			
≤5 years	108(61)	19(17.6)	**0.009**
>5 years	69(39)	24(34.8)	
No of young breast cancer patients seen per month			
≤10	110(62.1)	25(22.7)	0.533
>10	67 (37.9)	18 (26.9)	
Awareness of international guidelines			
Aware or know where to access	131 (74)	37 (28.2)	**0.039**
Unaware	46(26)	6(13)	
Specialty of respondent			
Medical/pediatric oncologists	141(79.7)	34 (24.1)	0.912
Other specialties	36(20.3)	9 (25)	

*FP discussion in >90% of the AYA male patients consulted. ;AYA Adolescent and young adult, FP fertility preservation

(P 07) IDENTIFYING CONCERNS OF ADOLESCENT AND YOUNG ADULT SARCOMA PATIENTS AND SURVIVORS FOLLOWING FERTIITY PRESERVATION

Victoria Wytiaz, MD[1]; Nina Jackson, MSW[2]; Chiu Yi Tan, MSW[3]; Daria Stelmak, BS[4]; Nick Iannarino, PhD[5]; Anao Zhang, PhD[6]; Erin Ellman, MSW[7]; Lindsey Herrel, MD, MS[8]; Molly Moravek, MD, MPH, MSCI[9]; Emily Walling, MD, MPHS[10]; Rashmi Chugh, MD[11]; Brad Zebrack, PhD[6], (1) University of Michigan, Department of Hematology/Oncology, Ypsilanti, MI, (2) 2University of Michigan School of Social Work, Department of Anthropology, Ann Arbor, MI, (3) School of Social Policy & Practice, University of Pennsylvania, Philadelphia PA, USA, Philadelphia, PA, (4) University of Michigan School of Medicine, Ann Arbor, MI, (5) University of Michigan-Dearborn, Department of Language, Culture and the Arts, Dearborn, MI, (6) Univsersity of Michigan School of Social Work, Ann Arbor, MI, (7) Rogel Cancer Center, University of Michigan, Ann Arbor, MI, (8) University of Michigan, Department of Urology, Ann Arbor, MI, (9) University of Michigan, Department of Obstetrics and Gynecology, Ann Arbor, MI, (10) University of Michigan, Department of Pediatric Hematology/Oncology, Ann Arbor, MI, (11) University of Michigan Rogel Cancer Center, Ann Arbor, MI

Objective: The risk of infertility in the adolescent and young adult (AYA) sarcoma population is recognized by oncology providers. As such, validated measures have been developed and adopted by many practices to better assess cancer-related reproductive concerns and meet the needs of AYA oncology patients, including access to fertility preservation (FP). However, less is known about the needs of such patients following FP, as the AYA group spans a wide age spectrum with many pursuing FP well before they consider parenthood. The aim of this study was to better elucidate the concerns of AYA sarcoma patients post-FP to inform current oncofertility practices.

Methods: AYA cancer patients (n=27) aged 12-25 years whose treatment conferred a risk of infertility were recruited through electronic health record query. Participants over 18 years were emailed or mailed copies of informed consent documents. Guardian consent/minor assent were sent to participants below the age of 18. Interviews consisted of administration of a battery of standardized instruments followed by a semi-structured interview via HIPAA-approved confidential video-conferencing software, or in person. Interviews were comprised of three sections (general cancer experience, goals and dreams for the future, experiences with FP). The local Institutional Review Board approved this overall study entitled “Understanding adolescent and young adult cancer patient utilization of and preferences for clinical fertility preservation and reproductive health services.” Authors deductively coded interviews for themes related to information needs, knowledge of treatment effects on fertility, and reproductive concerns. 27 total participants were identified with7 participants having a diagnosis of sarcoma. Emergent, inductive themes related to post-FP concerns were identified from their interviews.

Results: 7 participants (3F, 4M) had a diagnosis of sarcoma (3 with synovial sarcoma, 3 with osteosarcoma and 1 with malignant peripheral nerve sheath tumor). Mean age of sarcoma participants was 18.3 years (range 13-24). All 7 patients attempted fertility preservation (6/7 successful) compared to 17/27 (64%) total participants who pursued FP. Uncertainty about family planning, feelings of precocity or forced maturity and concerns regarding future relationships emerged as common themes. Some participants expressed their struggle in accepting the unknown in terms of their ability to naturally reproduce. Others remarked that their sarcoma diagnosis mandated discussions regarding fertility that their peers could not appreciate. Similarly, some felt that they were too young to consider FP and this concern was

present in participants of varied ages (16-, 20-, 22- and 24-year-old participants). Multiple participants worried that the risk of infertility and their history of FP would complicate future romantic relationships.

Conclusion: AYA sarcoma patients are receptive to discussions regarding infertility risk and in this single-institution study, they do employ FP strategies at a rate higher than their peers. However, following even successful FP, fertility-related concerns persist and in exploring these themes, opportunities to strengthen current AYA oncofertility practices emerge. The themes identified in this study suggest a benefit to long-term AYA oncofertility-specific peer programs to provide both practical advice and emotional support Peer support programs in the AYA oncofertility space often emphasize FP options and associated risks and benefits. Encouraging AYA oncology patients in the survivorship setting to engage in such groups and share their experiences of FP and beyond would be beneficial as well. Similarly, current educational efforts regarding FP focuses on the decision-making process and logistics. Developing post-FP educational materials that target patients and families as well as providers of survivorship would bridge the gap between the time of FP and the time of family planning for many AYA sarcoma patients.

(P 08) **PRELIMINARY CLINICAL ACTIVITY OF THE TYPE II RAF INHIBITOR TOVORAFENIB IN RAF FUSION-DRIVEN RECURRENT/PROGRESSIVE SARCOMAS**

Natasha van Eijkelenburg, MD[1]; Jasper van der Lugt, MD, PhD[1]; Daniel Da Costa, PharmD[2]; Peter Manley, MD[3]; Stephanie Hume, PhD[2]; Lisa M. Kopp, DO, MPH[2]; Jeeyun Lee, MD[4], (1) Princess Máxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands, (2) Day One Biopharmaceuticals, Brisbane, CA, (3) Day One Biopharmaceuticals, Norfolk, MA, (4) Sungkyunkwan University School of Medicine, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Objective: BRAF and CRAF/RAF1 (known collectively as “RAF”) fusions are rare oncogenic alterations in sarcoma. Tovorafenib is an investigational, oral, selective, CNS-penetrant, type II RAF inhibitor with clinical activity in BRAF-fusion pediatric low-grade glioma (pLGG) and adult melanoma. Early clinical activity of tovorafenib monotherapy in two adolescent and young adult (AYA) patients─one with an inoperable, locally advanced BRAF-fusion inflammatory myofibroblastic tumor (IMT) and another with a CRAF-fusion spindle cell sarcoma─ as of 26 May 2023 is reported.

Methods: Two ongoing phase 2 studies are evaluating tovorafenib monotherapy: FIREFLY-1 (FF-1) (PNOC026; NCT04775485), in pediatric patients (6 months to 25 years of age) with recurrent/progressive RAF-fusion advanced solid tumors in arm 3, and sub-study DAY101-102a of FIRELIGHT-1 (FL-1) (NCT04985604), in patients ≥12 years of age with RAF-fusion or CRAF/RAF1-amplified solid tumors. In both trials, tovorafenib 420 mg/m2 (maximum 600 mg) is administered PO once weekly in 28-day cycles until disease progression and the primary endpoint in solid tumors is overall response rate per RECIST version 1.1.

Results: Patient 1 is a 15-year-old female with a locally advanced pelvic IMT harboring an ETV6-BRAF fusion. Surgical resection of the large tumor was contraindicated due to high morbidity risk. She enrolled in Arm 3 of FF-1 and treatment was initiated at 600 mg once weekly. After 2 cycles of treatment, a 16% reduction (investigator-assessed) in the target lesion was reported and the tumor was deemed operable. Treatment continued for 3 more weeks (2.5 cycles total), and the tumor was resected with R0 margins. As the risk of relapse was low,

tovorafenib was discontinued post-surgery. No significant surgically-related morbidities were reported. TRAEs included G2 myalgia, fatigue, and headache that resolved following a reduction in tovorafenib dose to 500 mg. Transient G1/2 maculopapular rash and G2 acneiform rash were observed and resolved with supportive care medications and the dose reduction. The patient also experienced G1 anorexia, anemia, periorbital and localized edema, skin discoloration, chest erythema, pyrexia, nausea, and vomiting. Patient 2 is a 30-year-old Asian female enrolled in FL-1 with metastatic undifferentiated spindle cell sarcoma harboring an APPL2-RAF1 fusion previously treated with radiation, multiple surgical resections, and 2 lines of systemic therapy (cisplatin + etoposide [best response of stable disease] followed by docetaxel + gemcitabine [best response of partial response {PR} but progressed after 6 cycles]). A PR was reported after 2 cycles of tovorafenib (SPPD decrease of 47% from baseline [BL] on CT imaging), with a deepening of response with continued treatment of 10 cycles at data cut off (SPPD decrease of 72% from BL on CT imaging). TRAEs included G1 acne, pruritus, and hypothyroidism, and G3 CPK elevation. A G3 left retinal hemorrhage occurred with a concomitant eye trauma on C2D27 which resolved after 5 weeks. Tovorafenib dose was interrupted and then reduced to 500 mg with no further recurrences.

Conclusion: Tovorafenib has clinical activity and a manageable safety profile in these two AYA patients with RAF fusion sarcoma. These reports provide additional evidence of the antitumor activity of tovorafenib in RAF fusion-driven tumors beyond pLGG Early tumor shrinkage minimizing potential morbidities facilitated curative tumor resection in one patient where surgical intervention was not previously possible and a continued rapid, durable tumor response in another. Genomic analysis of all sarcomas is warranted to identify rare oncogenic gene fusions, especially when such alterations are targetable. Both the FF-1 and FL-1 trials are ongoing and continue to enroll.

(P 09) A NOMOGRAM MODEL TO PREDICT THE OVERALL SURVIVAL OF ADOLESCENT AND YOUNG ADULT PATIENTS WITH LOCALLY ADVANCED EWING SARCOMA.

Paulina Jagodzińska-Mucha[1]; Sylwia Kopeć, n/a[1]; Magdalena Rosińska, n/a[2]; Pawel Sobczuk, MD PhD[3]; Katarzyna Kozak, MD, PhD[4]; Paweł Teterycz, n/a[5]; Iwona Ługowska, n/a[6]; Anna raciborska, n/a[7]; Katarzyna Bilska, n/a[7]; Michał Wągrodzki, n/a[8]; Piotr Rutkowski, MD, PhD[9], (1) Department of Soft Tissue, Bone/Sarcoma, and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland., Warsaw, Mazowieckie, Poland, (2) Department of Computational Oncology, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland., Warsaw, Mazowieckie, Poland, (3) Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (4) Maria Sklodowska-Curie National Research Institute of Oncology Warsaw Poland, Warsaw, Mazowieckie, Poland, (5) Department of Soft Tissue, Bone/Sarcoma, and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland. Department of Computational Oncology, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland., Warsaw, Mazowieckie, Poland, (6) Early Phase Clinical Trials Unit, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland., Warsaw, Mazowieckie, Poland, (7) Department of Oncology and Surgical Oncology for Children and Youth, Mother and Child Institute, 01-211 Warsaw, Poland., Warsaw, Mazowieckie, Poland, (8) Department of Pathology and Laboratory Diagnostics, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland., Warsaw, Mazowieckie, Poland, (9) Maria Sklodowska-Curie National Research Institute of Oncology in

Warsaw, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Mazowieckie, Poland

Objective: There are several well-known prognostic factors for Ewing sarcoma (ES) patients, but so far they have not been implemented in decision-making in clinical practice. We aimed to construct a prognostic model for overall survival after the completion of the curative treatment in patients with locally advanced ES.

Methods: We extracted data on 256 nonmetastatic patients ≤25 years of age, treated between 1998 and 2018 at Mother and Child Institute, Warsaw (n=192) and Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw (n=64). raining set (n=188) was used to identify independent prognostic factors for overall survival (OS) with the Kaplan-Meier estimator, log-rank test, and the multivariate Cox model. Identified factors were used to construct the nomogram, which was validated in the validation set (n=65).

Results: The results of multivariate Cox regression used to create the nomogram showed that age, pathologic fracture, type of induction chemotherapy (VIDE vs other), type of local treatment (radiotherapy+ surgery vs surgery vs radiotherapy), and the time of diagnosis were independent prognostic factors. Pathological fracture (HR 4.62, 95% CI 1.81 – 11.80), and no local treatment (6.10, 95%CI 1.93 – 19.30) are negative prognostic factors. The prognosis improved significantly with the time of diagnosis, by 8% per year (HR 0.92, 95% CI 0.87-0.97). Age 10 years or less at diagnosis and receiving VIDE as an induction therapy regimen tended to predict better OS. Model calibration assessment showed that the nomogram has a good agreement for the patient's survival at selected time points (24 and 60 months). The Harrell's concordance index on the training dataset was 0.78 (95%CI 0.72 - 0.84) and on the validation dataset i0.69 (95% CI 0.56 – 0.83).

Conclusion: Our nomogram has predictive power and could be helpful to assess the risk among ES patients and guide treatment decisions; however, it should be further validated in external datasets.

(P 10) **ATEZOLIZUMAB IN JAPANESE PATIENTS WITH ADVANCED ALVEOLAR SOFT PART SARCOMA: A MULTI-CENTER, SINGLE-ARM, INVESTIGATOR-INITIATED PHASE 2 TRIAL (NCCH1907)**

Makoto Endo, MD, PhD[1]; Yuki Kojima, MD, Ph.D[2]; Motoko Arakaki, M.D.[3]; Tadaaki Nishikawa, M.D., PhD.[3]; Yukari Hoshina, R.Ph, MS[3]; Kenta Anjo, n/a[3]; Ryunosuke Machida, ME[3]; Akihiro Hirakawa, PhD.[4]; Masahiko Ichimura, MS[3]; Hitomi S. Okuma, MD, PhD.[3]; Kenichi Nakamura, M.D., Ph.D., M.B.A.[3]; Ikuo Kudawara, MD, PhD.[5]; Masanobu Takahashi, M.D., PhD.[6]; Kan Yonemori, MD, Ph.D[2], (1) Kyushu University, Fukuoka, Fukuoka, Japan, (2) Department of Medical Oncology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (3) National Cancer Center Hospital, Tokyo, Tokyo, Japan, (4) Tokyo Medical and Dental University, Tokyo, Tokyo, Japan, (5) Osaka National Hospital/National Hospital Organization , Osaka, Osaka, Japan, (6) Tohoku University Hospital, Sendai, Miyagi, Japan

Objective: Alveolar soft part sarcoma is a rare and clinically indolent soft tissue sarcoma subtype. Here, we report the clinical activity and safety of atezolizumab, an anti-PD-L1 immune checkpoint inhibitor, in Japanese patients with unresectable, recurrent, or metastatic alveolar soft part sarcoma.

Methods: In this study, patients were enrolled from 4 hospitals in Japan. Eligible patients eligible were aged 16 years or older with histologically confirmed, locally advanced or metastatic alveolar soft part sarcoma; an Eastern Cooperative Oncology Group performance status score of 0-1; and adequate bone marrow, hepatic, and renal function. Patients received 1200 mg of atezolizumab every three weeks until disease progression, unacceptable toxicity, or patient refusal. The primary endpoint was the objective response rate (ORR) measured according to the Response Evaluation Criteria in Solid Tumors, version 1.1, evaluated by blinded independent central review. Secondary endpoints were progression-free survival (PFS), overall survival (OS), and safety. This trial is registered with jRCT, number 2031200041.

Results: Between May 2020 and March 2021, 21 patients with alveolar soft part sarcoma were enrolled in the study and deemed eligible for inclusion. The median patient age was 33 years (range, 18-61 years). Fourteen patients (66.7%) received prior systemic treatment; 13 patients received pazopanib. Twenty patients were evaluable for the primary endpoint. The overall ORR was 10.0% (95% confidence interval (CI), 1.2%-31.7%). Complete responses were observed in 2 patients (10.0%), and response continued for more than 1 year. Stable disease was present in 14 patients (70.0%). The disease control rate was 80.0% (95% CI, 56.3%-94.3%). The median PFS was 10.0 months [95% CI, 3.3-16.7] and the median OS was not reached [95% CI, 14.9-not estimable (NE)]. The most common treatment-related adverse events were fever (40.0%) and lymphocyte count decreased (30.0%). The most frequent grade 3 or worse adverse events were lymphocyte count decreased (three [15.0%]), CPK increased, white blood cell count decreased, weight gain, fever, parotitis, anemia, thyroiditis, cancer pain and liver function abnormalities (one [5.0% each]). There were no treatment-related deaths.

Conclusion: Atezolizumab showed modest antitumor activity with manageable toxicities for Japanese patients with unresectable, recurrent, or metastatic alveolar soft part sarcoma.

(P 11) LX-101, A NOVEL, CLINICAL STAGE, PAYLOAD-BEARING TARGETED THERAPY DIRECTED TO THE INSULIN-LIKE GROWTH FACTOR RECEPTOR, HAS POTENT PRECLINICAL ANTI-TUMOR ACTIVITY AGAINST PEDIATRIC SARCOMAS

Matthew Hoberman, BSE, Lirum Therapeutics, New York, NY

Objective: LX-101, a next-generation, targeted therapy directed to the insulin-like growth factor 1 receptor (IGF-1R), consists of a proprietary IGF-1 variant coupled to a cytotoxic methotrexate (MTX) payload. LX-101 was previously evaluated in Phase 1 trials of adult patients with advanced, pretreated cancers, where it was well-tolerated and demonstrated single agent activity. Neither a dose limiting toxicity, nor a maximum tolerated dose were reached, leaving potential room for additional dose escalation and schedule optimization. Several aggressive pediatric sarcomas of unmet need have genetic alterations affecting the IGF-1R pathway and/or have high IGF-1R expression that correlates with poor outcomes, including Ewing's sarcoma, rhabdomyosarcoma, osteosarcoma, and others. Prior attempts at inhibiting the IGF-1R with non-payload bearing naked monoclonal antibodies or small molecule tyrosine kinase inhibitors produced a range of clinical outcomes in Ewing's and other related sarcomas, including some partial and complete responses. None of these agents, however, were ultimately approved in an oncology setting, potentially due to suboptimal potency that allowed for redundant signaling pathways and other escape mechanisms. Here, we investigated the anti-tumor activity of the payload-bearing agent LX-101 against pediatric sarcoma cell lines in vitro.

Methods: Cell lines were incubated with LX-101 (~1.6 - 2500nM). Cell viability was assessed by CellTiter-Glo after 4 days. IC50s were calculated using GraphPad PRISM software.

Results: The results are summarized in Table 1. All Ewing's sarcoma cell lines tested were sensitive to LX-101, including EWSR1-FLI1 gene fusion-positive cell lines RD-ES (IC50 = 10 nM), A-673 (IC50 = 14 nM), and SK-ES-1 (IC50 = 29 nM) and EWSR1-ERG gene fusion-positive cell line CADO-ES1 (IC50 = 14 nM). LX-101 was also potent against PAX3-FOXO1 gene fusion positive alveolar rhabdomyosarcoma (SJCRH30, IC50 = 23 nM) and osteosarcoma (143B, IC50 = 6 nM; HOS, IC50 = 7 nM; U2OS, IC50 = 32 nM).

Conclusion: These results demonstrate that LX-101 has potent preclinical anti-tumor activity against pediatric sarcoma cell lines with well-established ties to the IGF-1R pathway, including with different oncogenic gene fusions. These data further support the clinical development of LX-101 in IGF-1R-related pediatric cancers. A clinical trial is planned.

Indication	Cell lines	Absolute IC_{50} (nM IGF)[2]
Ewing's sarcoma	RD-ES	10
	CADO-ES1	14
	A673	14
	SK-ES-1	29
Rhabdomyosarcoma	SJCRH30	23
Osteosarcoma	143B	6
	HOS	7
	U2OS	32

(P 12) MULTIFOCAL AND DISSEMINATED SPORADIC DESMOID TUMOUR OF THE EXTREMITIES: A SPECIAL CHALLENGE

Franka Menge[1]; Peter Hohenberger, N/A, n/a[1]; Bernd Kasper, MD, PhD[2]; Eva Wardelmann, MD[3]; Florian Haller, N/A, n/a[4]; Jens Jakob, MD[5], (1) Sarcoma Unit, Department of Surgery and Mannheim Cancer Center, University Medical Center Mannheim, University of Heidelberg, Mannheim,Germany, Mannheim, Baden-Wurttemberg, Germany, (2) University of Heidelberg, Mannheim University Medical Center, Mannheim, Baden-Wurttemberg, Germany, (3) Institute of Pathology,.University Hospital Muenster, Muenster, Nordrhein-Westfalen, Germany, (4) Department of Pathology Friedrich- Alexander- Universität Erlangen-Nürnberg, Erlangen,

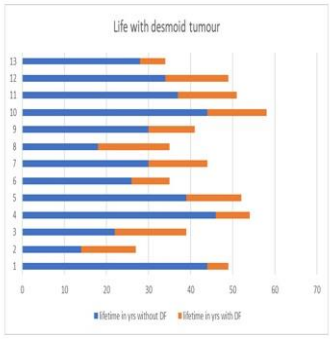
Erlangen, Bayern, Germany, (5) University Medical Center Mannheim, University of Heidelberg, Mannheim, Baden-Wurttemberg, Germany

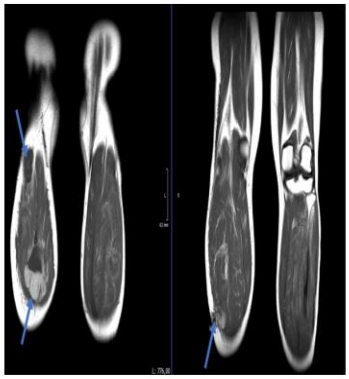
Objective: Desmoid tumour (DT) (aggressive fibromatosis) is a rare, non-metastatic but often locally aggressive tumour. DT mainly occur in young adults during the time of settlement, starting professional career and having family plans. Most DT exhibit a diffuse, but unifocal growth pattern. Some DT grow multifocal or disseminated which makes treatment even more challenging. Scant knowledge exists about the mechanism and impulsion of this scattered growth or the adequate therapy for these pts. Here we describe the clinical course and treatment of a series of 13 patients with multifocal/ disseminated DT.

Methods: From our prospective kept database with 392 females and 179 males we could identify 502 pts with a sporadic desmoid tumour. 13 (1,4%) patients suffered from a multifocal/ disseminated disease

Results: 11/13 pts. were female. The mean age was 32 years at time of diagnosis. Only 1 of 13 DT was located at the upper arm, 12 at the leg/ gluteal and 3 DT involved the small pelvis. In 5 (39%) pts the DT was multifocal/disseminated at time of diagnosis, while in 7 (54%) pts a spread of the disease occurred after surgery (1 pt. unknown). In 12 pts. a CTNNB1 mutation analysis was done and revealed a typical CTNNB1 mutation in all cases, confirming the diagnosis of sporadic DT (4 pts T41A, 7 pts S45F, 1 pt S45P). The maximum number of different treatments was 8 (with an average of 4,1). 11/13 (85%) pts had at least one receptor tyrosine kinase inhibitor (RTKI) as systemic treatment. 11/13 pts had at least one operation and 7/13 (54%) a radiotherapy with a dose of up to 60Gy. Only 1 pt. suffers from minor burden while the rest is significantly limited in the daily routine and spare time due to ongoing pain (13/13 pts. 100%), contracture (3/13 pts. 23%) or difficulty while sitting and walking (11/13 pts. 85%). Mean time of follow up is 11 yrs. (3-17yrs). 6 pts. are currently in stable disease.

Conclusion: Multifocal and disseminated DT is rare in patients with sporadic DT -however, disease burden is high with almost all patients suffering from chronic pain and relevant limitations in mobility. A multifocal growth pattern may be associated with previous resections but also occurs in previously untreated cases. The mechanism of spreading is still unknown. No standard treatments are available, most patients in our cohort received systemic treatment with RTKI to achieve local control.


Life with desmoid tumour
13
12
11
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
70
Lifetime in yrs without DF
Lifetime in yrs with DF



(P 13) OUTCOME OF PATIENTS WITH RELAPSED OR REFRACTORY NON-RHABDOMYOSARCOMA SOFT TISSUE SARCOMAS ENROLLED IN PHASE II COOPERATIVE GROUP CLINICAL TRIALS: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP

Sapna Oberoi, MD[1]; Amira Qumseya, n/a[2]; Wei Xue, PhD[3]; Rajkumar Venkatramani, MD[4]; Aaron R. Weiss, DO[5], (1) CancerCare Manitoba, Winnipeg, MB, Canada, (2) University of Florida, Gainesville, FL, (3) University of Florida, Gainsville, FL, (4) Baylor College of Medicine / Texas Children's Hospital, Houston, TX, (5) Maine Children's Cancer Program, Scarborough, ME

Objective: Nearly 25% to 30% of children and young adults with non-rhabdomyosarcoma soft tissue sarcomas (NRSTS) suffer a relapse or progression following first-line treatment. No clinical trials have solely focused on treating children and young adults with relapsed or refractory NRSTS; thus, data on outcomes for this population are lacking. The primary objective of our analysis was to estimate the event-free survival (EFS) of children and young adults with relapsed or refractory NRSTS treated in single-arm phase II studies conducted by the Children's Oncology Group (COG) and predecessor groups to establish a benchmark EFS as a primary endpoint for future phase II NRSTS trials. The secondary objectives were to compare the EFS by the pre-defined patient- disease- and trial-related characteristics and type of radiographic response and estimate the overall survival (OS).

Methods: A retrospective analysis of recurrent or refractory NRSTS patients enrolled in single-arm phase II COG, Pediatric Oncology Group and Children's Cancer Group trials between 1999 and 2015 was conducted. We used the Kaplan-Meier Method to estimate EFS and OS. The EFS was defined as disease progression/relapse or death. The Log-rank test was used to compare the EFS distribution between prespecified subgroups based on age at enrollment, sex, race/ethnicity, NRSTS histology, prior lines of therapy, calendar year of trial and type of radiographic response. A two-sided p-value < 0.05 was considered statistically significant.

Results: Our analysis included 137 patients [median age: 17 years (range: 1.6-26.9)] enrolled in thirteen phase II trials. All trials used response rate as the primary endpoint incorporating either WHO or RECIST radiographic response criteria. Of the 121 patients with evaluable radiographic response, 96 (70.1%) had progressive disease (PD), 22 (16.1%) had stable disease (SD), 3 (2.2%) achieved complete response (CR) while no patient had a partial response. None of the agents used in the trials were considered active based on the trial-specified response rates. The median duration of follow-up for the patients that were event-free at the last follow-up was three years. The estimated 6-month EFS of the entire study cohort was 19.4% [95% Confidence Interval (CI): 12.7%-26%)] (Figure 1). No significant difference in 6-months EFS was observed by age at enrollment [<=15 years vs. > 15 years (24.1% vs. 18.2%)], race/ethnicity [Black or African American vs. other vs. White (47.6% vs. 22.2% vs. 15.3%)], sex [female vs. male (17.6% vs. 20.4%)], histology subtype [synovial sarcoma (22%, 95% CI: 9.3% to 34.6%), desmoplastic small round cell tumor (18%, 95% CI: 0% to 37.9%) and malignant peripheral nerve sheath tumors (19.1%, 95% CI 7.9% to 30.4%)], by prior lines of therapies [1 or 2 lines vs. 1 or more lines (18.9% vs. 19.5%)] and by trial initiation year [before year 2006 vs. after year 2006 (23.2% vs.15.2%)]. The 6-month EFS significantly differed by the type of radiographic response (PD vs. SD vs. CR: 11.5% vs. 47.8% vs. 33.3%, P= < 0.0001). The 1-year and 2-year OS of the whole study cohort was 33.8% (95% CI: 25.5% to 42%) and 16.1% (95% CI: 8.9% to 23.4%), respectively (Figure 2).

Conclusion: The EFS and OS for children and young adults with relapsed or refractory NRSTS

remain suboptimal. We have established baseline EFS outcomes that can be referenced as a benchmark for future single-arm phase II trials incorporating potentially active novel agents in this population.

Figure 1. Event-free survival of NRSTS patients enrolled in thirteen cooperative group phase II clinical trials

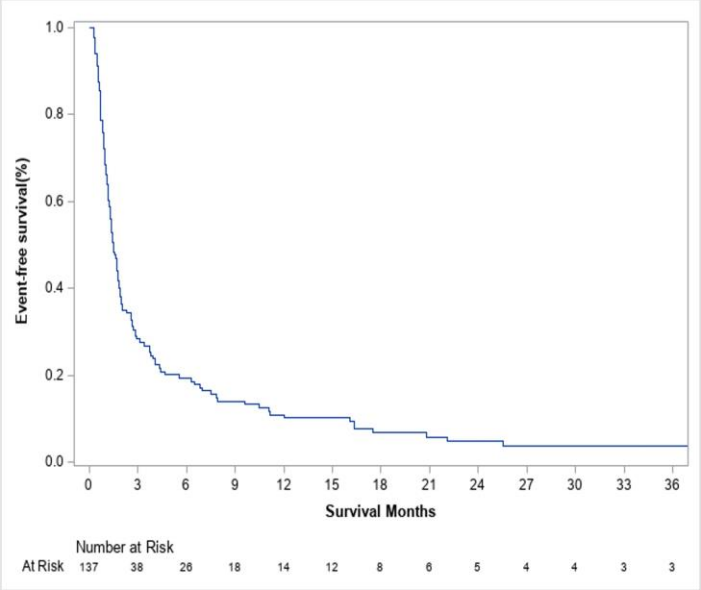
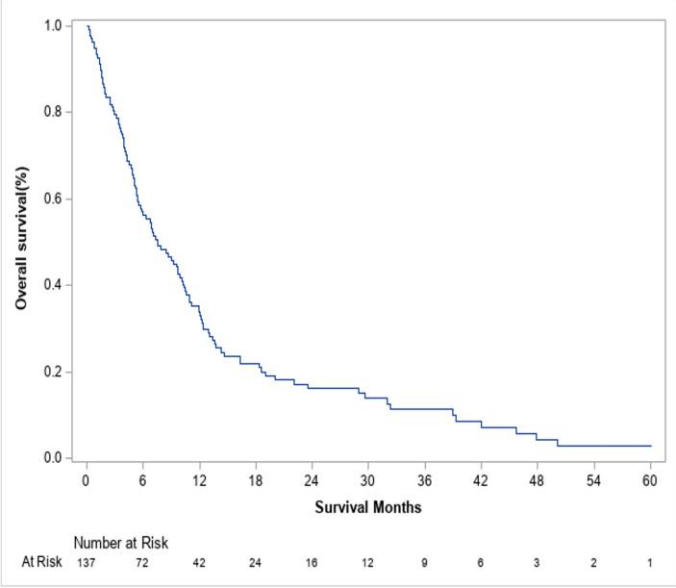


Figure 2. Overall survival of the NRSTS patients enrolled in the thirteen cooperative group phase II clinical trials



(P 14) **PERCUTANEOUS CRYOABLATION OF PROGRESSING EXTRA-ABDOMINAL DESMOID TUMOURS**

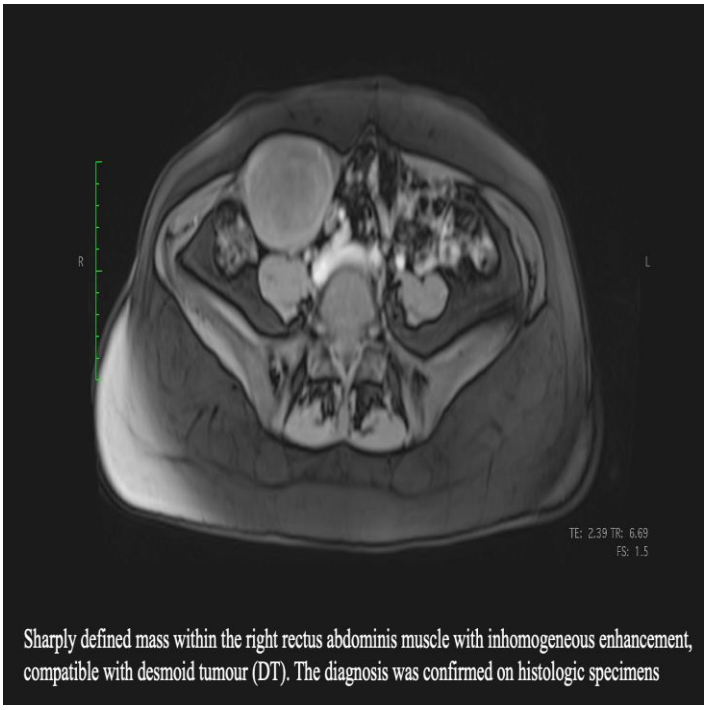
Andrea Vanzulli, MD[1]; Lucilla V. Sciacqua, MD[2]; Tommaso Cascella, MD[3]; Carlo Spreafico, M.D.[3]; Chiara Colombo, MD[4]; Elena Palassini, N/A, MD[5]; Alessandro Gronchi, MD, FSSO[6]; Carlo Morosi, MD[7], (1) Università degli Studi di Milano, Tradate, Lombardia, Italy, (2) Università degli Studi di Milano, Milan, Lombardia, Italy, (3) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (4) Department of Surgery, Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (5) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (6) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (7) Department of Radiology, Fondazione IRCSS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy

Objective: To evaluate the feasibility and efficacy of percutaneous cryoablation of extra-abdominal desmoid tumours progressing after first-line treatments.

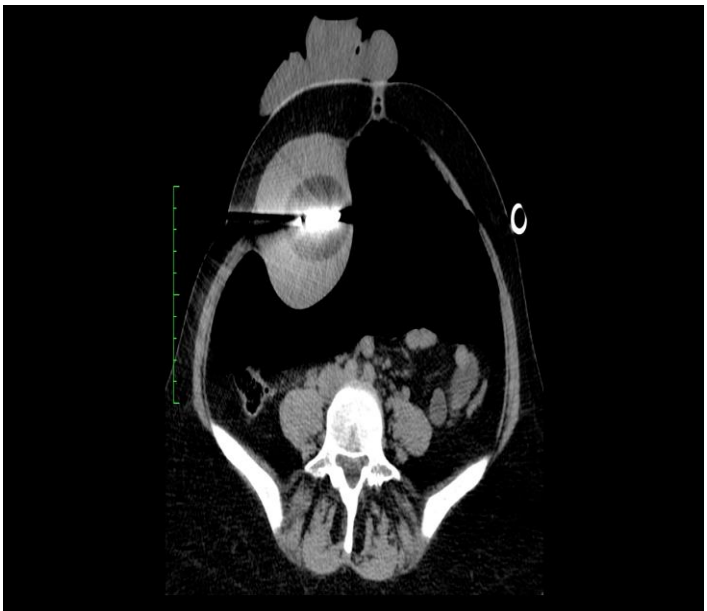
Methods: We retrospectively evaluated baseline and post-treatment MRI and/or CT imaging of all consecutive patients with histologically confirmed progressing extra-abdominal desmoid tumours treated with percutaneous cryoablation at our Institution between May 2021 and November 2022.
Treatment response was assessed both with standard and modified RECIST 1.1 criteria. Modified RECIST 1.1 criteria evaluate viable tumour alone, defined as such by the uptake of contrast agent in the arterial phase of dynamic imaging at either CT or MRI, with the underlying premise that dimensional reduction alone does not adequately capture tissue viability and may therefore incompletely depict responses to local treatments.
The variables of our analysis were disease progression, treatment safety and alleviation of pain and disability.

Results: The study cohort comprised 19 patients (17 females and 2 males), with a median age of 36 years (range 13-59 years), in radiological and/or clinical progression after first-line treatments (14 patients were enrolled in active surveillance, 3 patients received chemotherapy and 2 patients had undergone surgery).
Ten patients were not eligible for the procedure because of the close proximity of the lesion to the skin surface and/or neurovascular bundles.
One patient was excluded from the analysis because developed local infection at the site of insertion of the cryoprobe and the presence of inflammatory alterations hampered the evaluation of treatment response.
Tumour locations included the head & neck (3), the trunk (3), the abdominal wall (11) and the lower extremities/girdle (2), with an average maximum diameter of 80,2 mm (range 40-125 mm).
Five out of nineteen patients didn't have adequate contrast-enhanced pre-treatment imaging and couldn't therefore be evaluated with modified RECIST 1.1 criteria.
No RECIST progressions were detected at a median follow-up time of 149 days (range 29-254 days).
According to standard RECIST 1.1 criteria, 16/19 (84,2%) patients showed stable disease (SD) and 3/19 (15,8%) patients displayed a partial response (PR). According to modified RECIST 1.1 criteria, 9/14 (64,3%) patients presented with SD, 4/14 (28,6%) patients with PR and 1/14 (7,1%) patient with a complete response (CR), with 2 SD patients being reallocated to PR and CR categories, respectively.
Of note, 84% of patients reported significant improvement of symptoms.

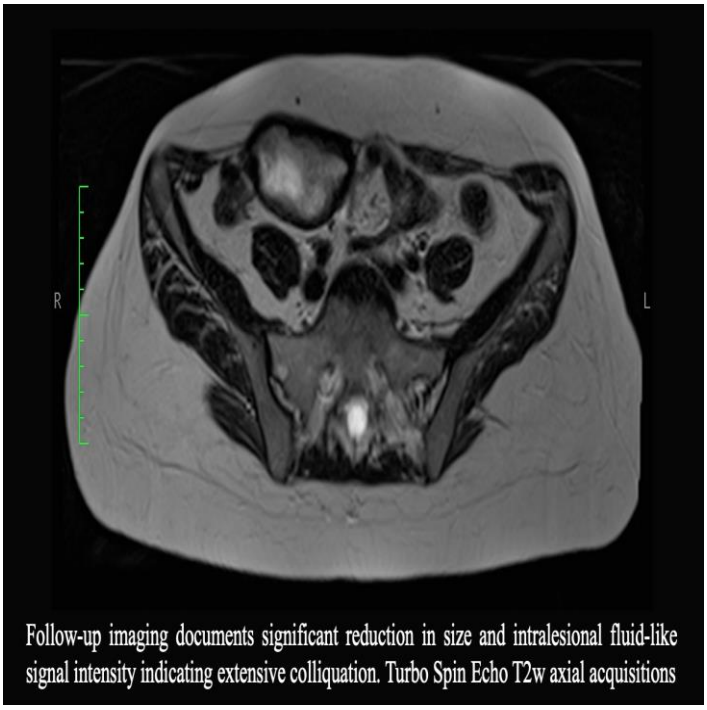
Conclusion: Our preliminary results further support the emerging role of percutaneous cryoablation for the treatment of progressing extra-abdominal desmoid tumours and highlight that modified RECIST 1.1 criteria outperform standard RECIST1.1 in this clinical scenario.



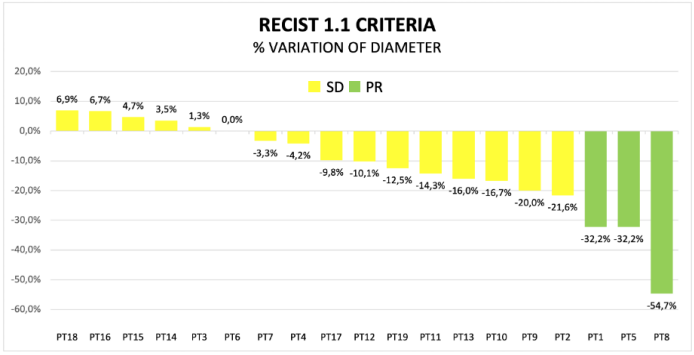
Sharply defined mass within the right rectus abdominis muscle with inhomogeneous enhancement, compatible with desmoid tumour (DT). The diagnosis was confirmed on histologic specimens

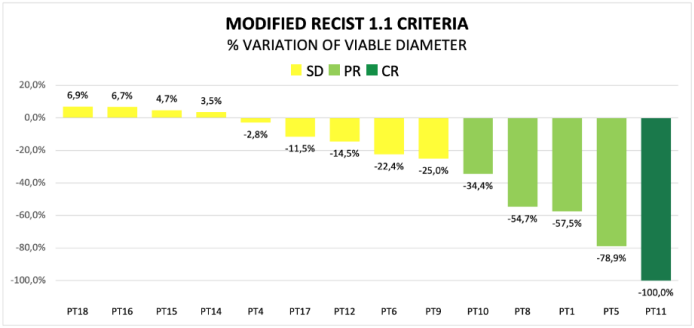


Four right-angle 3,8 mm cryoprobes and four freeze-thaw cycles with pull-back technique. Iatrogenic pneumoperitoneum was created to distance bowel loops from treatment area, and removed after reduction of the ice-ball to minimize heat-shock damage



Follow-up imaging documents significant reduction in size and intralesional fluid-like signal intensity indicating extensive colliquation. Turbo Spin Echo T2w axial acquisitions





(P 15) INTRAOSSEOUS RHABDOMYOSARCOMA OF THE CHEST WITH FUS:TFCP2 FUSION AND GERMLINE VARIANTS IN NF1 AND SBDS: NOVEL LOCATION AND MOLECULAR CHARACTERISTICS OF A RARE TUMOR

Amber Brown, DO, MS; Marissa Just, MD; Michael J. Ferguson, MD, MS; Mark S. Marshall, PhD, MS; Kari Bjornard, MD, MPH, Division of Pediatric Hematology/Oncology, Riley Hospital for Children, Indianapolis, IN

Objective: Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma in children and is occasionally associated with cancer predisposition syndromes such as neurofibromatosis type 1 (NF1). Intraosseous rhabdomyosarcoma (IRMS) is a recently introduced, rare subtype of spindle cell RMS that shows predilection for the craniofacial bones of female adolescents and young adults (AYAs) and has a characteristic gene fusion of transcription factor cellular promoter 2 (TFCP2) with Ewing sarcoma RNA binding protein 1 (EWSR1) or fused in sarcoma (FUS). Less commonly, IRMS has been found in the femur and pelvis. Here we present a case of IRMS of the chest wall in an adolescent with NF1 that on imaging was originally thought to be a malignant peripheral nerve sheath tumor. To the best of our knowledge, this is only the second reported case of IRMS of the chest wall, first in an adolescent, and the first reported case of IRMS in NF1.

Methods: Next generation sequencing (NGS) was performed by Caris Life Sciences, which includes tumor whole exome and transcriptome sequencing along with detection of copy number variants, insertions/deletions, loss of heterozygosity, tumor mutational burden, and microsatellite instability. Germline testing was performed by Prevention Genetics NGS and included exome sequencing and copy number variant detection.

Results: A 13-year-old female with NF1 presented with fatigue, right upper extremity pain, and unintentional weight loss. Labs showed leukocytosis, normal hemoglobin, and thrombocytosis. Computed tomography revealed a right upper chest mass (10.5 x 9.9 x 10.3 cm) with extension into the neck and spinal canal, metastatic pulmonary lesions, and pathologic compression fractures of the first and second thoracic vertebrae. Bilateral bone marrow specimens prior to therapy initiation were negative for sarcoma but showed hypocellular marrow. Primary lesion biopsy showed epithelioid and spindle cells immunopositive for desmin, MYOD1, ALK, and H3K27ME3. Fluorescence in situ hybridization revealed FUS rearrangement. Molecular tumor profiling revealed FUS:TFCP2 fusion (FUS exon 6 joined in-frame to TFCP2 exon 2) and pathologic NF1 variant (c.5595dup), confirming IRMS. VAC (vincristine, dactinomycin, cyclophosphamide) therapy was initiated. After two doses of dactinomycin, she developed grade 3 veno-occlusive disease (VOD) of the liver, a rare complication of dactinomycin. She was successfully treated with defibrotide and transitioned to VIE (vincristine, ifosfamide, etoposide) given comparable response and toxicity to VAC in RMS. She developed prolonged cytopenias on VIE, prompting repeat marrow studies, which were hypocellular but remained negative for sarcoma. Inherited bone marrow failure syndrome testing was performed given cytopenias and hypocellular marrow on initial and repeat specimens and showed germline pathogenic/likely pathogenic heterozygous variants in Schwachman-Bodian-Diamond syndrome (SBDS) (c.258+2T>C) and NF1 (c.5595dup). Pancreatic isoenzymes were negative. To the best of our knowledge, she is a carrier but does not have Schwachman-Diamond syndrome. She is still undergoing therapy but has responded well to VIE and radiation, with partial response of the primary lesion and complete response of the metastatic lesions by the Response Evaluation Criteria in Solid Tumors.

Conclusion: This is the first reported case of IRMS in association with NF1 and of the chest

wall in an adolescent. Reported cases of RMS and NF1 are almost exclusively embryonal RMS, with coexistence of IRMS and NF1 not previously reported in the literature. Germline pathogenic variants in SBDS were only recently linked to RMS, but have not been reported in conjunction with NF1 variants, and their clinical significance is still uncertain. Our patient's development of VOD with VAC therapy is uncommon and questions the impact of the collection of these rare genetic variants on therapy tolerability. This case supports the use of molecular tumor profiling paired with germline analysis to better delineate tumor pathology and to begin to understand complicated host biology. Further investigation is warranted into the clinical significance of the heterozygous SBDS variant in relation to NF1, RMS susceptibility, and risk of toxicity with chemotherapeutic agents commonly used to treat RMS.

(P 16) ALVEOLAR SOFT PART SARCOMA: PROGRESS TOWARD IMPROVEMENT IN SURVIVAL? A NATIONWIDE DATABASE STUDY IN JAPAN

Tomohiro Fujiwara, MD, PhD[1]; Toshiyuki Kunisada, n/a[1]; Eiji Nakata, MD[1]; Toshifumi Ozaki, MD[1]; Akira Kawai, MD, Ph.D[2], (1) Okayama University Hospital, Okayama, Okayama, Japan, (2) National Cancer Center Hospital, Tokyo, Tokyo, Japan

Objective: Alveolar soft part sarcoma (ASPS) is a rare histological subtype of soft-tissue sarcoma, which remains refractory to conventional cytotoxic chemotherapy. We aimed to characterize ASPS and investigate whether the oncological outcome has improved over the past decade.

Methods: One hundred and twenty patients with newly diagnosed ASPS from 2006 to 2017, identified from the Bone and Soft-Tissue Tumor Registry in Japan, were analyzed retrospectively.

Results: The study cohort comprised 34 (28%) patients with localized ASPS and 86 (72%) with metastatic disease at presentation. The 5-year disease-specific survival (DSS) was 68% for all patients and 86% and 62% for localized and metastatic disease, respectively ($p = 0.019$). Metastasis at presentation was the only adverse prognostic factor for DSS (hazard ratio [HR]: 7.65; $p = 0.048$). Patients who were >25 years (80%; $p = 0.023$), had deep-seated tumors (75%; $p = 0.002$), and tumors >5 cm (5–10 cm, 81%; >10 cm, 81%; $p < 0.001$) were more likely to have metastases at presentation. In patients with localized ASPS, adjuvant chemotherapy or radiotherapy did not affect survival, and 13 patients (45%) developed distant metastases in the lung (n = 12, 92%) and brain (n = 2, 15%). In patients with metastatic ASPS (lung, n = 85 [99%]; bone, n = 12 [14%]; and brain n = 9 [11%]), surgery for the primary or metastatic site did not affect survival. Prolonged survival was seen in patients who received pazopanib treatment ($p = 0.045$), but not in those who received doxorubicin-based cytotoxic chemotherapy. Overall, improved DSS for metastatic ASPS has been observed since 2012 (5-year, from 58% to 65%) when pazopanib was approved for advanced diseases, although without a statistically significant difference ($p = 0.117$).

Conclusion: The national study confirmed a unique feature of ASPS with frequent metastasis to the lung and brain but an indolent clinical course. An overall trend toward prolonged survival after the introduction of targeted drugs encourages continuous efforts to develop novel therapeutic options for this therapeutically resistant soft-tissue sarcoma.

(P 17) METASTASIS-SPECIFIC GENE EXPRESSION PATTERNS IN EWING SARCOMA

Maximilian Kerkhoff, MSc[1]; Pauline Plaumann, B.Sc. Biology[1]; Hatice Önder, n/a[1]; Christiane Schaefer, PhD[1]; Stefan K. Zöllner, MD[1]; Wolfgang Hartmann, N/A, MD[2]; Uta Dirksen, MD[3], (1) Pediatrics III, University Hospital Essen, West German Cancer Center, Essen, Nordrhein-Westfalen, Germany, (2) University Hospital Münster, Münster, Nordrhein-Westfalen, Germany, (3) University Hospital Essen, Pediatrics III, West German Cancer Center, German Cancer Research Center (DKTK) site Essen, National Center for Tumordiseases (NCT) site Essen, ,, Essen, Nordrhein-Westfalen, Germany

Objective: Prognosis for Ewing Sarcoma (EwS) patients with disseminated disease or at relapse remains poor. In most cases, the site of relapse corresponds to the site metastases. Though it is speculated that the transient reduction of oncogene expression promotes dissemination, the mechanisms of metastasis in EwS remain elusive. Therefore, we aimed to identify differentially expressed genes specific for metastases.

Methods: We re-evaluated published microarray datasets on gene expression levels of primary, metastatic and recurrent tumor samples, healthy tissue samples and cell lines. We checked whether the resulting hits were directly regulated by EWSR1-FLI1 by published RNA-Seq data and qRT-PCR after EWSR1-FLI1 knockdown. Furthermore, the 27 highest expressed hits were tested in qRT-PCRs for four EwS cell lines and two human mesenchymal stem cell (hMSC) lines to provide a basis for in-vitro studies to reverse-engineer metastatic mechanisms.

Results: Our data analysis revealed 72 upregulated genes in EwS metastases. One of the top hits was the cancer testis antigen PRAME. Interestingly, most upregulated genes (68%) were also highly expressed in EwS cell lines. These results were confirmed by qRT-PCRs for the top hits in four EwS cell lines, so far. While 36 upregulated genes were downregulated, only one gene (HOXB13) was upregulated upon EWSR1-FLI1 knockdown in an RNA-Seq dataset.

Conclusion: Most of the upregulated genes we found in EwS metastasis data have already been associated with migration, invasion or aggressiveness in other cancer entities. We are currently analyzing whether PRAME expression is associated with severity of the disease in histological patient samples. Strikingly, only HOXB13 seemed to be negatively regulated by the fusion oncogene, rendering it a putative mediator in metastasis. We will evaluate our findings by analyzing the impact of manipulating the expression levels of some of the top hits in vitro and in vivo.

(P 18) RETROSPECTIVE ANALYSIS ON OUTCOMES PEDIATRIC PATIENTS WITH SARCOMA AND LUNG METASTASES STATUS POST PNEUMONECTOMY VERSUS PARTIAL LUNG RESECTION

Simran Jatar, MPH[1]; Amy Frantz, ACPNP[2]; Holly J. Meany, MD[2]; Marie V. Nelson, MD[2]; Mikael Petrosyan, MD, MBA[2]; AeRang Kim, MD, PhD[2], (1) Children's National Hospital/Georgetown University School of Medicine, Washington, DC, (2) Children's National Hospital, Washington, DC

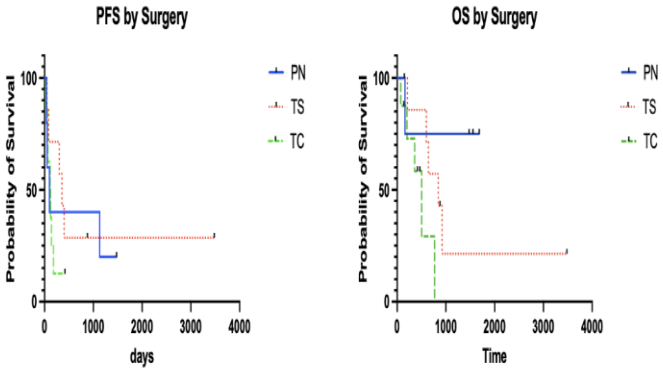
Objective: Complete resection has been shown to be an important favorable prognostic factor after pulmonary resection in osteosarcoma and soft tissue sarcoma. This retrospective analysis reviewed the outcomes of pediatric patients with sarcoma and lung metastasis who underwent pulmonary nodular surgery for lung metastases. There is limited data showing the efficacy and morbidity among pediatric patients who have received pneumonectomies for definitive pulmonary nodule resection. The goal of this study was to determine the difference between the type of pulmonary resection procedures, namely thoracoscopy, thoracotomy, and pneumonectomy in pediatric sarcoma patients with pulmonary metastases and their impact on acute complications, disease recurrence, and survival.

Methods: Retrospective data was collected from a single tertiary center to investigate the outcomes of pediatric patients with a primary diagnosis of sarcoma and pulmonary metastases who underwent pulmonary surgery at this institution between 2010 and 2023. Primary tumors in the lung or chest were excluded. For those with more than one pulmonary surgery, only the first surgery was included unless they had a pneumonectomy. Relevant information pertaining to the included patients was collected from medical records and electronic databases. This includes but is not limited to demographic data, primary tumor characteristics, metastatic disease details, surgical procedures performed, postoperative outcomes, and long-term follow-up data.

Results: Twenty patients (14 M, 6 F) with the median age at diagnosis being 13y (2-24) and surgery being 15y (range 7-24) were included. Diagnoses were osteosarcoma (n=12), rhabdomyosarcoma, Ewing sarcoma, high-grade sarcoma (n=2 each), synovial sarcoma, and MPNST (n=1 each). Most primary tumors were located in the extremity (13 lower extremity, 6 upper extremity, and one central location). One-third (n=7) had lung metastases at diagnosis and all were treated with upfront chemotherapy, surgery, and/or radiation. The pulmonary surgical summary is listed in Table 1. All patients who underwent pneumonectomy (n=5) had unilateral disease, nodules larger than 1 cm, and hilar location. Patients who underwent thoracotomy (n=8) mostly had bilateral disease (88%) whereas those who underwent thoracoscopy (n=7) mostly had unilateral disease (85%). Those who underwent pneumonectomy had the greatest blood loss at the time of surgery, but their length of stay was not substantially different than those who underwent thoracotomy. In the total group, the median progression-free survival (PFS) from the time of surgery was 132 days and overall survival (OS) was 770 days. No differences were seen in PFS between the three groups, however, the OS was improved in the group who underwent pneumonectomy.

Conclusion: Surgical resection of metastatic disease in sarcoma can provide disease control for patients. This analysis was limited by the small numbers at a single institution to do a true comparison analysis. Our cohort of pediatric patients was able to undergo pneumonectomy safely without significant complications compared to other lung surgeries traditionally used for pulmonary nodule resection and may provide another modality used in certain clinical scenarios to render patients surgically disease free that may benefit long-term outcomes.

Figure 1. Progression Free and Overall Survival Curves by Surgical Type



Abbreviations: PN- Pneumonectomy; TS- Thoracoscopy; TC- Thoracotomy; PFS- progression free survival; OS- overall survival

Table 1. Characteristics of Pulmonary Nodule Resection by Surgical Type

Type of Surgery	Pneumonectomy (n=5)	Thoracoscopy (n=7)	Thoracotomy (n=8)
Location of nodules			
Unilateral	5	6	1
Bilateral	0	1	7
Number of Nodules			
Solitary	2	5	1
Multiple	3	2	7
Size			
> 1 cm	5	1	4
< 1 cm	0	6	4
Average (SD) Estimated blood loss*	230 (192)	5 (9)	69 (98)
Median (range) Length of hospital Stay	5 (2-8)	2 (1-6)	8 (3-15)

*minimum blood loss calculated as zero

(P 19) EXTRACELLULAR VESICLE CLICK MAGBEADS: A MULTIFACETED LIQUID BIOPSY PLATFORM FOR PEDIATRIC SARCOMAS

Steven J. Jonas, MD/PhD; Junseok Lee, PhD; Diego S. Banuet Gonzalez; Yusef Mathkour; Kenny Vo, MD; Hadar Keren-Gill, MD; Sarah M. Dry, N/A, MD; Noah Federman, MD; yazhen Zhu, MD/PhD; Hsian-Rong Tseng, PhD, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA

Objective: There is a pressing need for developing new diagnostic tools for the pediatric sarcoma community that enable more precise and timely detection of a patient's disease progression prior to radiographic evidence of treatment failure or disease recurrence. Using osteosarcoma (OS) as a clinical model, we demonstrate a liquid biopsy approach capable of simultaneously assessing systemic tumor burden via isolation of tumor-derived extracellular vesicles (EVs) and quantifying the activity of proteases used by OS to promote tumor invasion and dissemination. Isolating and characterizing sarcoma-derived EVs for specific protease activity profiling is expected to offer an opportunity for precision cancer diagnosis and treatment monitoring.

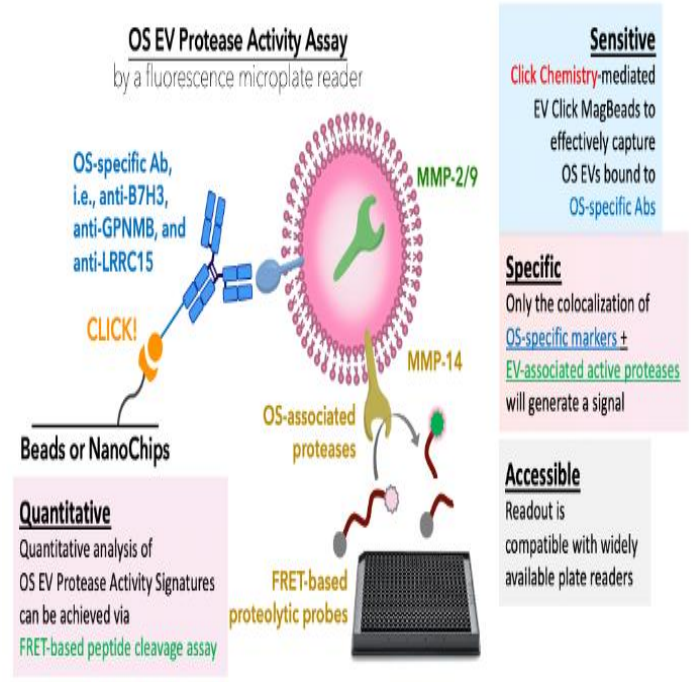
Methods: Given the crucial role of EV-mediated transport of active proteases in tumor progression, we hypothesized that profiling OS EV protease activity signatures can be utilized to detect disease progression in OS patients. To evaluate this hypothesis, we developed and synergistically integrated two platform technologies (Figure 1): i) EV Click MagBeads for OS EV enrichment -that leverage click chemistry-mediated targeting of three OS-specific markers (i.e., B7H3, GPNMB, and LRRC15); and ii) Fluorescence resonance energy transfer (FRET)-based peptide cleavage assays that quantify the activity of OS-associated active proteases, including MMP-14 and MMP-2/9, which play a crucial role in modulating OS progression and metastasis. The detection capabilities of this integrated OS EV Protease Activity liquid biopsy platform were validated in both artificial plasma samples and in samples obtained from a small OS patient cohort (n=30) undergoing treatment at the UCLA Pediatric Bone and Soft Tissue Sarcoma Program.

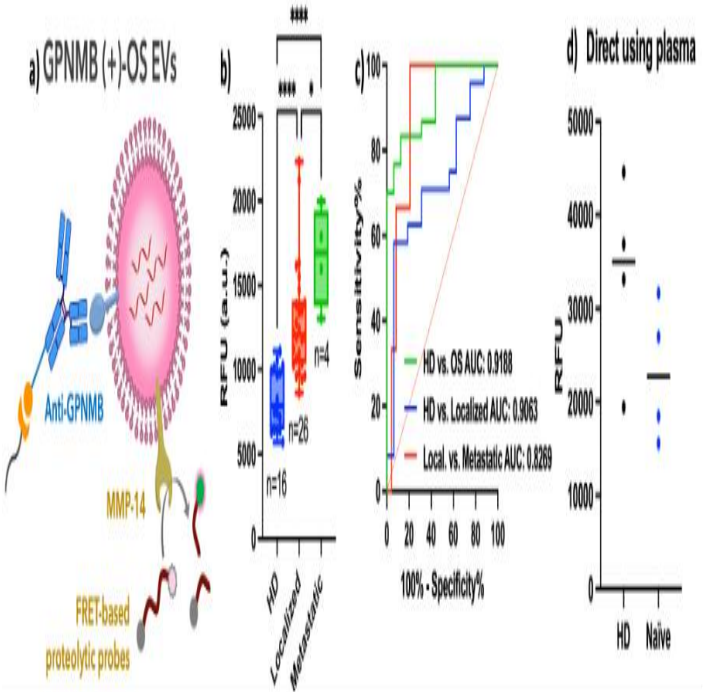
Results: The concentration dependence of the OS EV protease activity of artificial plasma samples was evaluated using GPNMB as an OS-specific marker for EV isolation. MMP-14 activity from captured GPNMB(+)-OS EVs was measured via the FRET-based peptide cleavage assay and a linear titration curve (R2 = 0.987) was observed. We next preliminarily assessed whether our OS EV MMP-14 Activity Assay (Figure 2) could differentiate OS patients with known metastatic disease from localized OS patients and healthy donors. Using MMP-14 as a representative OS-associated active protease, we observed elevated activity in OS EV samples obtained from the metastatic OS patients, represented by the AUROC of 0.92 (healthy vs. localized OS) and 0.83 (localized vs metastatic OS). No significant difference was observed between the whole plasma from healthy donors and OS patients without EV enrichment.

Our preliminary studies further applied the OS EV Protease Activity assay to determine whether dynamic changes in protease activity could be correlated to treatment response. Serial samples taken before the initiation of treatment and up to 9 weeks after the first blood draw were assessed from two OS patients. Radiographic findings from routine surveillance imaging from these patients are consistent with the dynamic changes observed in the corresponding MMP-14 activity on GPNMB(+)-OS EVs.

Conclusion: Liquid biopsy of sarcoma-derived EVs represents a promising approach to systemically and repeatedly assess the activity of key proteases of the tumor, aiding in the

diagnosis, prognostication, and detection of disease progression. This multifaceted OS EV Protease Activity liquid biopsy assay is rapidly translatable, offering pediatric oncologists a sensitive noninvasive solution for dynamic monitoring of treatment response and detecting disease progression in OS patients. This capability could enable pediatric oncologists to make more informed and effective alterations to their management quickly and with higher confidence.





(P 20) CLEAR CELL SARCOMA: PATIENT REPORTED REAL WORLD EVIDENCE

Lennie D. Woods[1]; Jill Neal, n/a[2]; Lisa A. Faulkner, BSN, RN[3]; Sara Woods, n/a[1]; Audrey Neal, n/a[4]; Jacqueline Woods, n/a[1]; Denise Reinke, MS, NP[5]; Kelley Argraves, PhD[6], (1) Clear Cell Sarcoma Foundation, Charleston, SC, (2) Clear Cell Sarcoma Foundation, Geneseo, IL, (3) Clear Cell Sarcoma Foundation, Austin, TX, (4) Clear Cell Sarcoma Foundation, Cincinnati, OH, (5) University of Michigan, Ann Arbor, MI, (6) Clear Cell Sarcoma Foundation, Johns Island, SC

Objective: Clear Cell Sarcoma (CCS) is an ultra-rare and aggressive soft tissue sarcoma which typically develops in tendons or aponeuroses, and affects mostly teens and young adults. In a majority of cases, CCS is caused by a translocation resulting in the fusion of the genes for activating transcription factor 1 (ATF1) and Ewing sarcoma breakpoint region 1 (EWSR1), resulting in the EWSR1-ATF1 fusion protein. Since CCS is so rare, there is a paucity of research, leading to few treatment options that are generally based on weak scientific evidence. The CCS patient community is eager to Share RWE data as reported by CCS patients and/or their primary caregiver with hopes to initiate further patient data collection and disease natural history research with clinicians, researchers and patients working together. Persons with CCS have been sharing their lived experiences with each other for more than ten years on a private Facebook page and want the sarcoma community to have access to that Real World data through this presentation for the purpose of improving patient outcomes.

Methods: An anonymous electronic survey was created using a basic questionnaire originally designed by the founding members of the CCS Family page along with additional Input from the Clear Cell Sarcoma Foundation (CCSF) medical advisory board. Survey respondents either participate in the CCS family private Facebook group (308 members) or have reached out to the CCSF through the website. The survey was administered via a HIPPA compliant platform called Survey Monkey (now “Momentive”). CCS patients and caregivers were given a dedicated email address to request the survey for participation. All responses are de-identified through the platform.

Results: Forty-two surveys were requested by email. Thirty-seven de-identified surveys were completed at the time of this submission.
Sixty percent of the participants were female. Eighty-three percent of respondents were under the age of 45 at diagnosis. Forty-Seven percent were under age 25 at diagnosis and many of the respondents had the “bump” for years prior to the diagnosis. Fifty-three percent had disease localized to extremities. Twenty-four percent were found to have metastatic disease at diagnosis. Fifty-five percent of patients had a visible lump prior to CCS being diagnosed and 60% had pain in the area of the tumor.
Eighty-four percent of misdiagnosed CCS patients were originally misdiagnosed with either Melanoma or Ewing sarcoma. The correct diagnosis for CCS is often made after the standard of care treatment for the wrong disease is given. This has a negative effect on the patient. Twenty-four percent of respondents found errors in their medical records.
Ninety-seven percent of clear cell sarcoma patients surveyed want to be involved in research and are willing to give their time and share information with researchers and clinicians who want to help find better diagnostic tests and treatments for CCS.

Conclusion: Many research projects put a high value on gathering medical data directly from patient’s charts or the treating institutions’ in an effort to minimize patient reported inaccuracies. Twenty-four percent of CCS patients report they are misdiagnosed or intentionally diagnosed with something other than CCS in a well-meaning effort to ensure insurance will allow the

treatment prescribed by the treating physician. The CCSF recommends patients and researchers collaborate on projects where CCS patient data is gathered to ensure accurate information is being used. This includes retrospective data.

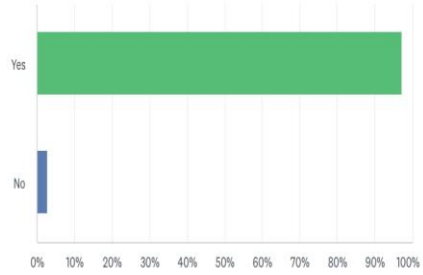
Blood work is a relatively quick and easy way for the general health of CCS patients to be monitored and for researchers to develop diagnostic assays to monitor disease and treatment response.

A majority of CCS patients had a lump and even more had pain in the area of the tumor before diagnosis. Sarcomas are generally thought of as not producing pain which can lead to misdiagnosis as a sprain or injury, thus prolonging proper treatment.

Nearly all of the patients queried responded positively about being involved in medical research related to their CCS diagnosis. The CCSF encourages collaboration and open discussions with the CCS family of patients. Please reach out by sending an email to info@clearcellsarcoma.org to set up a focus group or discuss how we can help each other.

Would you, the caregiver or person with CCS, be willing to participate in medical or research style focus groups alongside other CCS caregivers and patients from time to time? You can opt out later.

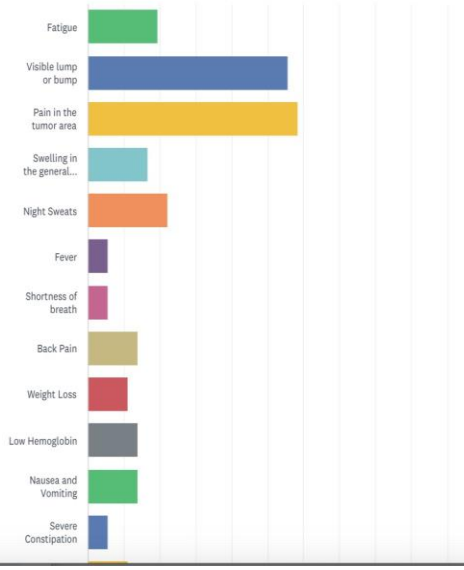
Answered: 35 Skipped: 2



ANSWER CHOICES	RESPONSES	
Yes	97.14%	34
No	2.86%	1
TOTAL		35

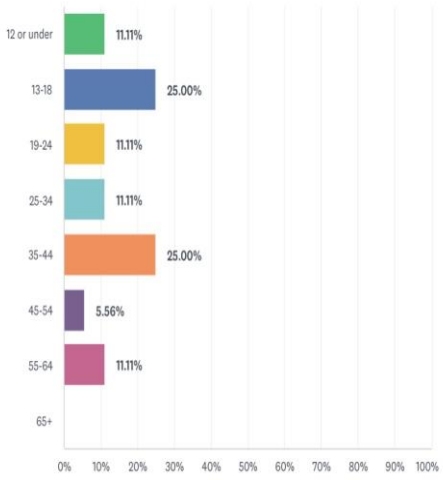
What were the symptoms of the person with CCS that led to seeking medical advice? Check all that apply.

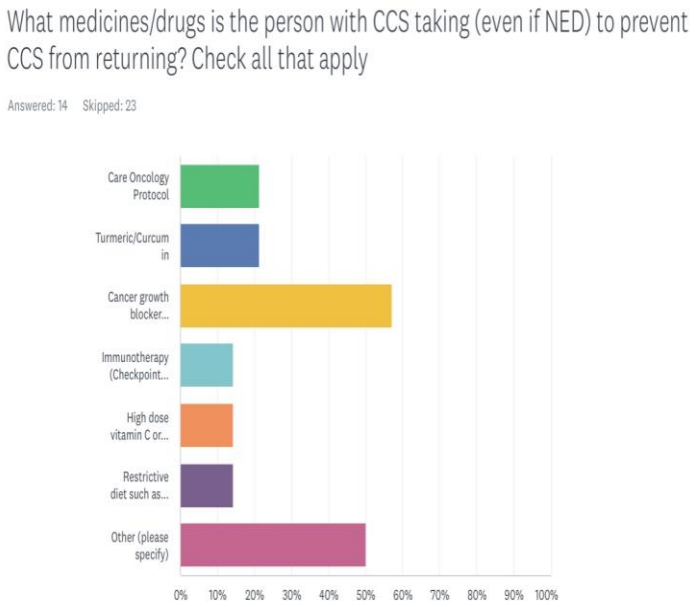
Answered: 36 Skipped: 1



At what age was person with CCS originally diagnosed with Clear Cell Sarcoma? (if the original diagnosis was incorrect, still use that age) A later question will be asked about this).

Answered: 36 Skipped: 1





(P 21) YOUNG ADULTS WITH NONMETASTATIC OPERABLE OSTEOSARCOMA TREATED PERIOPERATIVELY WITH METHOTREXATE, DOXORUBICIN AND CISPLATIN SCHEDULE, SINGLE INSTITUTION REPORT

Joanna M. Placzke, MD[1]; Aneta W. Borkowska, MD, PhD[2]; Tomasz Switaj, MD, PhD[3]; Pawel Rogala, MD PhD[3]; Anna Mariuk-Jarema, MD[4]; Sylwia Kopeć, n/a[5]; Michal Wagrodzki, MD, PhD[4]; Hanna Koseła-Paterczyk, MD PhD[6]; Piotr Rutkowski, MD, PhD[3], (1) Maria Sklodowska-Curie National Research Institute of Oncology, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Poland, WARSAW, Mazowieckie, Poland, (2) Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (3) Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Mazowieckie, Poland, (4) Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland, Warsaw, Mazowieckie, Poland, (5) Department of Soft Tissue, Bone/Sarcoma, and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland., Warsaw, Mazowieckie, Poland, (6) Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland

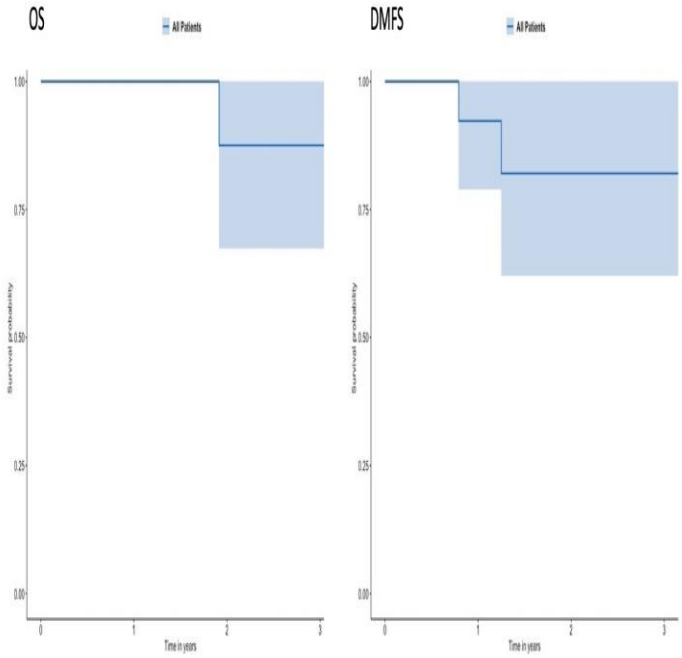
Objective: Osteosarcoma has its peak incidence in adolescents and young adults (AYA) between 16 and 30 years however this young group of patients is underrepresented in clinical trials and in analyses concerning survival prognosis. Treated with three or more drug schedules (including Methotrexate-MTX, Doxorubicin and Cisplatin; MAP) designated for children, AYA do not have the same outcomes and reliable prognostic markers providing relevant estimation of prognosis.

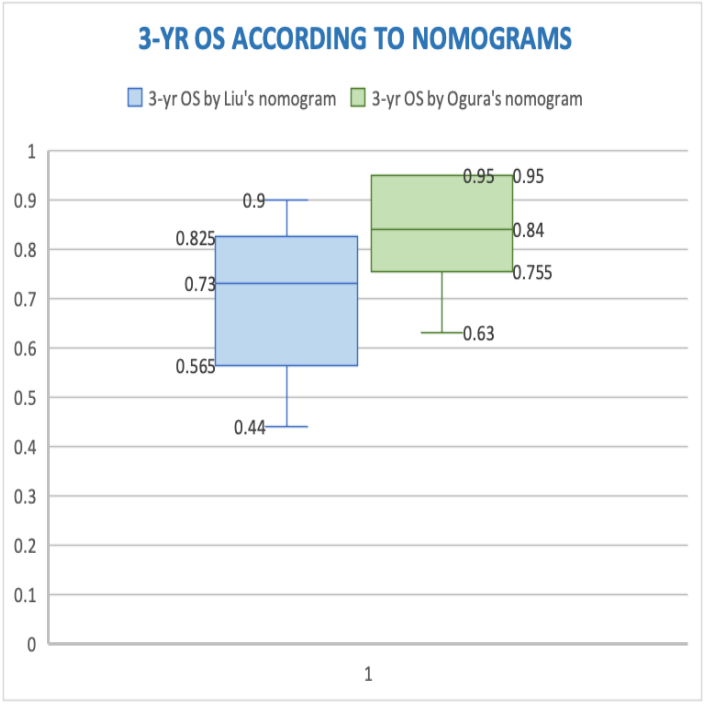
Methods: Treated in our department and eligible for the analysis were patients (pts): in the age of 18-30 inclusively, diagnosed with non-metastatic high-grade operable skeletal osteosarcoma,

received at least one dose of methotrexate preoperatively as a part of MAP schedule. Data on patient, disease, treatment and survival characteristics were retrospectively collected. OS (overall survival) and DMFS (distant metastases free survival) for the analyzed group were calculated using “R” software.
Survival predictions using validated Koichi Ogura’s Nomogram of 2015 and Ziege Liu’s Nomogram of 2023 were carried out for each patient based on the variables included in nomograms. We looked at pts in our dataset who had an event during the follow-up and compared their data with those with worse prognosis according to nomograms but with better outcomes in our cohort. We took under consideration variables additional to those included in nomograms such as: clinical stage, presence of positive lymph nodes, dosages of chemotherapeutics (given pre- and postoperatively), osteosarcoma histology and alkaline phosphatase concentration at the treatment start. Descriptive statistics was performed due to a small sample size.

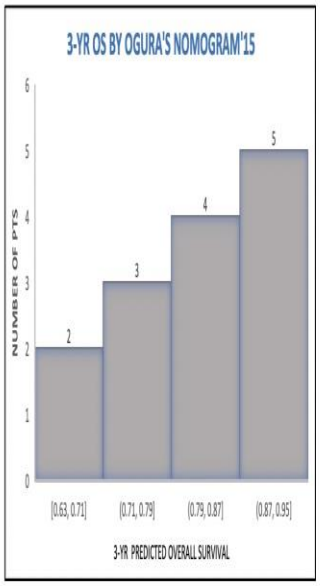
Results: Eligibility criteria were met by 16 pts treated between 2018-2023. Two of them were excluded due to the ongoing treatment. Median age was 22 (range: 19-25). Most of the pts were males (64%). Extremity was a primary tumor site in 71% of pts, while other were axial locations (rib, clavicle or pelvis). Three pts (21%) had pathological fracture. The most common stage by AJCC (The American Joint Committee on Cancer) was IIB (64%). With a median follow up of 42.3 months (range 7.9-60) we have observed relapses in two pts and one death. All pts received doxorubicin plus cisplatin pre and postoperatively, 28.6% (4) of pts did not receive MTX postoperatively, 78.6% (11) received all 4 doses of MTX preoperatively. There were no treatment-related deaths. Nobody relapsed during the treatment. One patient with no evidence of a disease during follow-up developed myelodysplastic syndrome 31 months after the end of treatment.
Calculated 3-year OS and DMFS rates in our cohort were 87.5% (95% CI: 0.673-1) and 82.1% (95% CI: 0.621-1), respectively.
Estimated mean of 3-yr OS for our pts by Liu's nomogram'23 was 69% and by Ogura’s nomogram’15 was 82%. Mean 3-yr DMFS estimated by Ogura’s nomogram’15 was 63%. Estimated mean for 3-yr OS and for DMFS in our pts according to Ogura’s and Liu’s nomograms were lower than actual calculated for the entire cohort.
We have observed that the deceased patient in our cohort did not have the worst survival prediction according to Liu’s and Ogura’s nomograms. Patients with better outcomes despite worse nomogram predictions for 3-yr OS in comparison to the deceased patient had: higher doxorubicin and cisplatin dosages per m2 for perioperative treatment, higher MTX dose per m2 per cycle preoperatively and lower clinical stage by AJCC, lower alkaline phosphatase concentration (less than 2.5 times the upper limit) and none lymph nodes involved.

Conclusion: Our findings, similarly to other studies, indicate that osteosarcoma in adolescents and young adults is not consistent with established prognostic factors for survival in other age groups. Distinctive analysis of prognostic factors in AYA group and nomograms created for them exclusively are needed. Statistical justification of possible impact on survival of such variables as: doxorubicin and cisplatin dosages per m2 for perioperative treatment, MTX dose per m2 per cycle preoperatively, alkaline phosphatase concentration of more than 2.5 times the upper limit at start and lymph node involvement is needed in much larger cohort and is planned for the future.

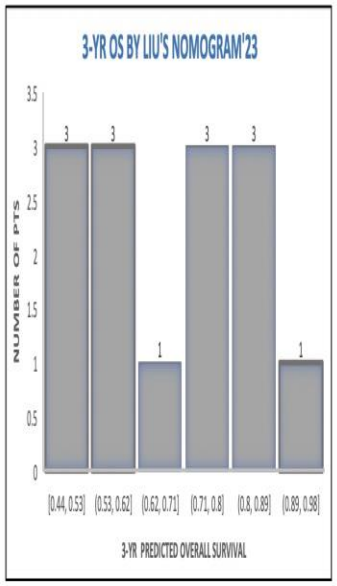

OS
All Patients
1.00
0.75
0.50
0.25
0.00
Survival probability
0
1
2
3
Time in years
DMFS
All Patients
1.00
0.75
0.50
0.25
0.00
Survival probability
0
1
2
3
Time in years


3-YR OS ACCORDING TO NOMOGRAMS
3-yr OS by Liu's nomogram
3-yr OS by Ogura's nomogram
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.9
0.825
0.73
0.565
0.44
0.95
0.95
0.84
0.755
0.63
1

A



B



	pts with worse nomogram prediction but better outcome in 3-yr **DMS** Ogura's Nomogram of 2015 had	pts with worse nomogram prediction but better outcome in 3-yr **OS** Ogura's Nomogram of 2015 had	pts with worse nomogram prediction but better outcome in 3-yr **OS** Liu's Nomogram of 2023 had	**might influence OVERALL SURVIVAL**
ADM/M2 in overall treatment	higher	higher	higher	+
CISPL/M2 in overall treatment	higher	higher	higher	+
MTX/M2 in overall treatment	-	-	-	
preoperative ADM/m2	-	-	-	
preoperative CISPL/m2	-	+	-	
preoperative MTXm2	-	-	-	
MTX/m2/CYCLE preoperatively	higher	higher	higher	+
CLINICAL STAGE by AJCC	-	lower	lower	+
CLINICAL STAGE by MSTS	-	lower	lower	+
N+	-	none	none	+
OSTEOSARCOMA histology	-	-	-	
ALKALINE PHOSPHATASE ABOVE LIMIT >2.5 times (N<=120)	lower	lower	lower	+

(P 22) RECALL EXPERIENCE FOR SURVIVORS OF PEDIATRIC RHABDOMYOSARCOMA TREATED AT A SINGLE INSTITUTION BETWEEN 1970 AND 2010

Karen J. Goddard, MD[1]; Jemma Say, MD[2]; Leigh-Ann Baron, NP[1]; Luminita Nica, MSc[1], (1) BC Cancer, Vancouver, BC, Canada, (2) National Health, Bath, England, United Kingdom

Objective: To assess long-term morbidity including the risk of second malignancy in survivors of pediatric rhabdomyosarcoma treated in British Columbia (BC) between 1970 and 2010 and lost to any ongoing follow-up at an oncology center.

Methods: We attempted to contact 43 adult survivors previously treated for rhabdomyosarcoma in BC in childhood (between the ages of less than 1 and less than 18) between 1970 and 2010. This effort was part of the BC Cancer Provincial Late Effects, Assessment and Follow-up (LEAF) Clinic Program for childhood and AYA cancer survivors to better serve cancer survivors. We contacted these patients using mail initially due to ethics board concerns regarding privacy. Many letters were returned due to incorrect address information. We managed to contact only 18 patients (16 patients could not be reached and appeared to be still living in BC, 1 patient declined to attend and 8 lived out of province). The results we have pertain to those patients who were assessed.

Results: Of the 18 patients assessed, 9 had received radiation therapy (RT) in addition to chemotherapy and 9 had received chemotherapy alone with surgery for local control. The average age at the time of therapy was 7.9 years. The average age at the time of first follow-up as an adult was 37 years. Of the 9 patients assessed who had received RT, all had multiple severe late effects including hypoplasia, psychological problems (anxiety and depression) infertility and second neoplasm (4 patients - 44% of this group - developed different secondary neoplasms: I radiation induced meningioma, one renal cancer and chronic lymphocytic leukemia, and 2 patients developed squamous cell carcinoma of the vulva - leading to the death of one patient 2 years after first recall appointment. The median time between diagnosis of initial disease and diagnosis of a second neoplasm was 38 years. Of the 9 patients who received only chemotherapy and surgery, only one patient developed second malignancies (bladder cancer diagnosed 38 years after initial therapy and papillary thyroid cancer diagnosed 46 years later). Two of the patients treated with chemotherapy alone had developed infertility, but no other significant long-term morbidities were noted in the chemotherapy and surgery alone patients.

Conclusion: We successfully contacted only 18 survivors of pediatric rhabdomyosarcoma in our Province and all of those previously treated with radiation therapy (RT) had very significant late effects related to their previous therapy. It is important when discharging these patients from active clinical oncology care to communicate their significant long-term health risks to their community medical team and to the patient In view of the ongoing, high risk of long-term side effects. It is also important to try to communicate with patients treated years ago with treatment regimens that often used RT so that their follow-up is approriate. Concerns regarding the patient's ongoing health care should override those of privacy and contact should be attempted using phone calls. These patients require life-long care informed by their previous treatment exposures.

Immunology-Immunotherapy

(P 23) GENOMIC COMPLEXITY AS A BIOMARKER FOR ADJUVANT IMATINIB TREATMENT IN HIGH-RISK GASTROINTESTINAL STROMAL TUMOR

Kjetil Boye, MD, PhD[1]; Heidi M. Namløs, PhD[1]; Ludmila Gorunova, PhD[1]; Ksenia Khelik, PhD[1]; Sigve Nakken, PhD[2]; Daniel Vodák, PhD[1]; Ivar Hompland, MD, PhD[1]; Bodil Bjerkehagen, MD, PhD[1]; Jeanne-Marie Berner, PhD[1]; Sverre Heim, MD, PhD[1]; Eivind Hovig, PhD[1]; Toto Hølmebakk, MD, PhD[3]; Ola Myklebost, PhD[4]; Francesca Micci, PhD[1]; Leonardo A. Meza-Zepeda, PhD[1], (1) Oslo University Hospital, Oslo, Oslo, Norway, (2) Oslo University Hospital, Nevlunghavn, Vestfold, Norway, (3) Oslo University Hospital, The Norwegian Radium Hospital, Oslo, Oslo, Norway, (4) University of Bergen, Bergen, Hordaland, Norway

Objective: Gastrointestinal stromal tumors (GISTs) are characterized by oncogenic KIT or platelet-derived growth factor alpha (PDGFRA) mutations, whereas malignant progression occurs through stepwise accumulation of additional chromosomal aberrations. Adjuvant imatinib treatment for three years is recommended for patients with localized GIST at high risk of recurrence. Nearly half of high-risk patients are cured by surgery alone and 30-40% relapse after stopping adjuvant therapy, indicating that the currently used risk classification does not precisely predict patient outcome after standard treatment. The aim of this study was to investigate if genomic tumor complexity could be used as a prognostic biomarker and to characterize in detail the genomic and transcriptomic landscape of high-risk GIST.

Methods: Patients who underwent resection of primary GIST at Oslo University Hospital between 1998 and 2020 were included. Karyotypes were categorized as simple if they had ≤ 5 chromosomal changes and complex if there were > 5 chromosomal aberrations. Somatic mutations, DNA copy number changes, and expression of mRNA and miRNA genes and pathways were investigated using whole exome sequencing, RNA sequencing, and miRNA expression analysis.

Results: Chromosomal aberrations were detected in 206 tumors, of which 76 had a complex karyotype. The most frequently observed changes were losses at 14q, 22q, 1p, and 15q. High-risk patients with a simple tumor karyotype had an estimated 5-year recurrence-free survival (RFS) of 94%, whereas patients with a complex karyotype had an estimated 5-year RFS of 51%. A complex karyotype was associated with poor RFS in multivariable analysis adjusted for the established prognostic factors tumor site, size, mitotic count, and rupture. The prognostic impact of genomic complexity was confirmed in an independent validation cohort. Using genomic and transcriptomic profiling in a subset of samples, we confirmed that chromosomal instability was the most pronounced genomic feature and associated with disease relapse. The tumor mutational burden was low; no recurrent actionable somatic mutations were identified except for mutations in KIT and PDGFRA. Gene expression analysis showed that mRNA and miRNA genes involved in cell cycle signaling and the kinetochore complex were associated with genomic complexity and differentially expressed between metastatic and non-metastatic tumors.

Conclusion: Genomic tumor complexity is an independent prognostic biomarker in localized, high-risk GIST and is associated with cell cycle deregulation and dysfunctional control of the kinetochore machinery. Recurrences were infrequent for tumors with simple karyotypes; hence,

de-escalation of adjuvant imatinib treatment should be explored in patients with genomically simple GISTs. Conversely, tumors with complex genomes frequently relapsed after adjuvant therapy. Patients with genomically complex tumors may benefit from continued imatinib treatment.

(P 24) COMPREHENSIVE ANALYSIS OF THE GENOMIC LANDSCAPE AND CLINICAL BEHAVIOR IN GIST USING NEXT GENERATION SEQUENCING (NGS, MSK-IMPACT)

Ciara M. Kelly, MBBCh[1]; Zhidong Gao, MD[2]; Karissa Whiting, MS[1]; Evan Seffar, MS[1]; francisco Sanchez-Vega, MD[3]; Cristina R. Antonescu, MD[1]; Satshil Rana, PhD[4]; Viswatej Avutu, MD[5]; Jason E. Chan, MD PhD[1]; Sandra P. D'Angelo, MD[1]; Mark A. Dickson, MD[3]; Mrinal Gounder, MD[1]; Mary Louise Keohan, MD[4]; Robert G. Maki, MD PhD[6]; Sujana Movva, MD[1]; evan rosenbaum, MD[4]; Edmund K. Bartlett, MD[1]; Aimee M. Crago, MD, PhD, FACS[1]; Brian Untch, MD[4]; Vivian E. Strong, MD[7]; Samuel Singer, MD, FACS[1]; mark ladanyi, MD[8]; david Solit, MD[4]; Li-Xuan Qin, PhD[1]; William D. Tap, MD[1]; Ping Chi, MD, PhD[1], (1) Memorial Sloan Kettering Cancer Center, New York, NY, (2) Department of Gastrointestinal Surgery, Peking University People‘s Hospital, Peking University, Peking, China, Peking, Beijing, China (People's Republic), (3) MSKCC, new york, NY, (4) MSKCC, New York, NY, (5) Memorial Sloan Kettering Cancer Center and the Weill Cornell College of Medicine, New York, NY, (6) Memorial Sloan Kettering Cancer Center; Professor, Weill Cornell Medical College, New York, NY, (7) mskcc, new york, NY, (8) mskcc, New York, NY

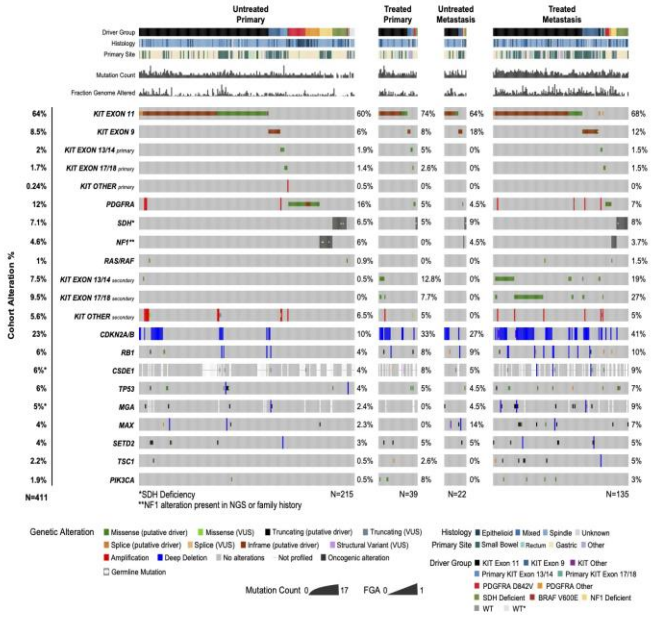
Objective: The relationship between the genomic profile in GIST and clinical behavior is relatively unknown. The objective of this study was to describe the genomic landscape in GIST and explore the association with tumor stage, treatment status and clinical outcome

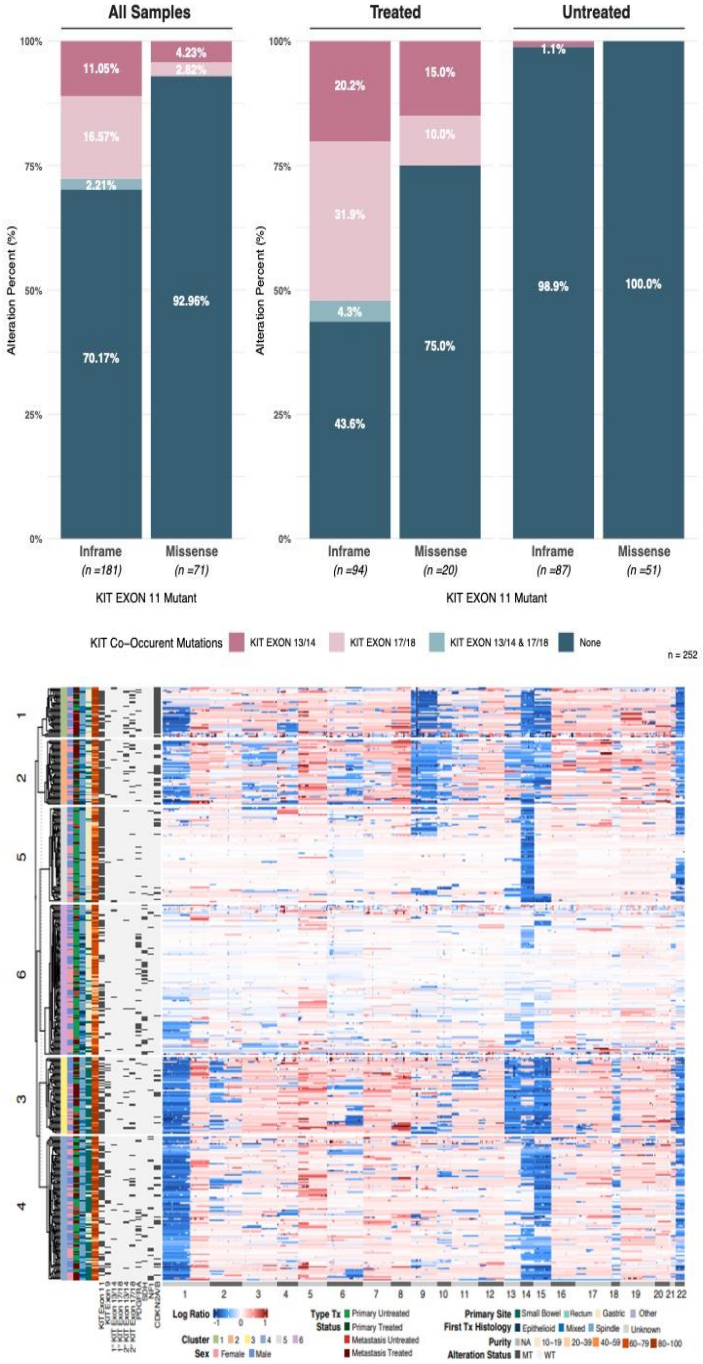
Methods: GIST patients (pts) that underwent NGS using MSK-IMPACT of archival tumor and matched normal DNA, in a prospective IRB-approved study were identified. Clinicopathologic data was collected, and a single sequenced sample was selected based on the most recent date of procurement and highest purity. Descriptive statistics were used to report clinicopathologic attributes, somatic, germline and copy number alterations (CNA), tumor mutational burden (TMB), and fraction of genome altered (FGA). Fishers exact, Chi-squared, or Wilcoxon rank sum tests, logistic regression and Kaplan-Meier analyses were performed as appropriate using R version 4.2.2.

Results: As of April 2020, 491 samples from 411 GIST patients were sequenced. (Table 1) The analysis included 254 primary tumor samples (15% treatment-exposed [TE]) and 157 metastatic/local recurrence samples (86% TE). Median (m)TMB was 1.80. mFGA was 0.19. NGS classified patients into six molecular subtypes [KIT- (76%; exon 11 - 64%, exon 9 - 8.5%, exon 13/14 - 2.4%, exon 17/18 - 1.7%), PDGFRα- (9.7%; D842V mutant- 5.8%), SDH-deficient (7.1%), NF1-related (4.6%), Wild type (1.9%), BRAF-mutant (0.2%)]. In KIT-mutant GIST (n=314) the most frequently altered genes included CDKN2A/B (29%), RB1 (8.0%), CSDE1 (7.0%), MGA (5.7%), SETD2 (5.4%), TP53 (5.1%), MAX (4.8%), TNFRSF14 (3.8%), PTEN (3.5%), GATA3 (3.2%), TERT (2.9%), TSC1 (2.9%), PDGFRA (2.5%), PIK3CA (2.5%). Additional gene mutations (muts) were infrequently observed in other subtypes except TP53 (17%) and TERT (10%) in SDH-deficient and CDKN2A/B (7.5%) in PDGFRα-mutant GIST. A significantly higher frequency of disruption in cell cycle, epigenetic, MYC and PI3Kinase pathways was observed in metastatic compared to primary tumor samples. Muts in CDKN2A/B,

RB1, TERT, MGA and TSC1 were significantly more common in TE samples. Co-occurring CDKN2A/B, MAX, TP53, and PIK3CA muts were associated with a poor prognosis in the metastatic setting. In KIT-mutant, TE samples, secondary KIT exon 13&14 and 17&18 alterations were observed in 19% % and 26% of patients. Secondary KIT muts significantly co-occurred with KIT exon 11-inframe deleted GIST but not -missense mutated GIST. In TE samples, KIT exon 17/18 muts (31.9%) were more common in inframe deleted GIST and exon 13/14 muts (15%) were more common in missense mutated GIST. (Fig. 2) Unsupervised clustering was done using CNA segmentation among the entire population. Six distinct genomic clusters emerged characterized by CNAs involving specific genes, clinicopathologic and molecular patterns. (Fig.3)

Conclusion: This study represents a one of the most comprehensive analyses of the genomic landscape and integration of genomic features with clinicopathologic attributes and survival outcome in GIST.




All Samples
Treated
Untreated
Alteration Percent (%)
100%
75%
50%
25%
0%
11.05%
16.57%
2.21%
70.17%
4.23%
2.82%
92.96%
20.2%
31.9%
4.3%
43.6%
15.0%
10.0%
75.0%
1.1%
98.9%
100.0%
Inframe
(n =181)
Missense
(n =71)
Inframe
(n =94)
Missense
(n =20)
Inframe
(n =87)
Missense
(n =51)
KIT EXON 11 Mutant
KIT EXON 11 Mutant
KIT Co-Occurent Mutations
KIT EXON 13/14
KIT EXON 17/18
KIT EXON 13/14 & 17/18
None
n = 252
Log Ratio
Type Tx
Primary Untreated
Status
Primary Treated
Metastasis Untreated
Metastasis Treated
Cluster
Sex
Female
Male
Primary Site
Small Bowel
Rectum
Gastric
Other
First Tx Histology
Epithelioid
Mixed
Spindle
Unknown
Purity
NA
10–19
20–39
40–59
60–79
80–100
Alteration Status
MT
WT

(P 25) PREDICTING IMATINIB RESPONSES USING IN VITRO MODELS OF PDGFRA-MUTANT GASTROINTESTINAL STROMAL TUMOR

Homma M. Khosroyani, B.S.[1]; Ajia Town, B.S.[1]; Johanna Falkenhorst, MD[2]; Sebastian Bauer, MD[2]; Michael C. Heinrich, MD[3], (1) OHSU Knight Cancer Institute, Portland, OR, (2) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (3) Portland VA Health Care System and Oregon Health & Science University Knight Cancer Institute, Portland, OR

Objective: Gastrointestinal stromal tumor (GIST) is the most common sarcoma with 4,000-6,000 annual cases diagnosed in the United States annually. The two most common GIST subtypes harbor oncogenic mutations in homologous receptor tyrosine kinases, KIT or platelet derived growth factor receptor alpha (PDGFRA), both of which are treated with tyrosine kinase inhibitors (TKIs). Type II TKIs like imatinib are successful in treating many patients with advanced KIT-mutant GIST, but are generally thought to be ineffective for PDGFRA-mutant GIST. Patients with the most common PDGFRA driver mutation, exon 18 D842V, historically had poor outcomes because their mutation is notoriously resistant to imatinib and other FDA-approved type II TKIs used for GIST. In 2020, the FDA approved avapritinib, a type I TKI, for the treatment of patients with advanced PDGFRA exon 18 mutant GIST. There are many other PDGFRA exon 18 mutations reported in GIST beyond D842V. We have curated data from a large cohort of PDGFRA-mutant GISTs, and over 80% of the mutations observed are in exon 18 (n=544), and include many unique non-D842V point mutations and in/dels (n=177/544). It is largely unknown how patients with these mutations respond to imatinib, however, currently most exon 18 mutations are assumed to be imatinib resistant like D842V. In previous work, we biochemically profiled some frequently observed patient mutations. Contrary to prior beliefs, we show that some of these exon 18 mutations were imatinib sensitive, and that sensitivity seemed to be dependent on the properties of the amino acid in the 842-codon position. Modeling each of the clinically reported mutations is not feasible, therefore based on the data above, we hypothesized that the imatinib sensitivity of PDGFRA exon 18 mutations could be predicted based on the 842 residue.

Methods: To test our hypothesis, we created mutagenic libraries in lentiviral vectors that included both point mutation as well as deletion mutation constructs that recapitulate patient mutations. Each mutation was expressed in Ba/F3 cells and underwent limiting dilution assays. From this, the oncogenic potential of each mutation along with imatinib sensitivity for –IL3 independent Ba/F3 cells was assessed using immunoblotting for phosphorylated-PDGFRA.

Results: In our assay, 13 out of the 20 amino acids when in the 842-codon position conferred imatinib sensitivity. This further debunks the belief that all PDGFRA exon 18 mutations are imatinib resistant. We found that the majority of hydrophobic residues (7 out of 8), conferred imatinib resistance (IC50 > 150nM), and amino acids from other classes (polar, negatively charged, positively charged, special case) were imatinib sensitive (IC50 < 150nM). Alanine, although hydrophobic, conferred imatinib sensitivity when at the 842-position. To understand the structural implications of these mutations on the kinase and on drug binding, we are currently doing molecular simulations and drug docking studies.

Conclusion: Our model has the potential to identify which PDGFRA exon 18 mutant GIST patients would benefit from front-line imatinib therapy, while subsequently identifying those that would require avapritinib treatment. Avapritinib was recently approved for first-line treatment for all PDGFRA exon 18 mutant GIST in the United States. Imatinib has been used clinically for over two decades, and is more cost-effective and has a superior safety and tolerability profile

compared to avapritinib. If a patient progresses on imatinib, there are additional FDA-approved treatments (sunitinib, regorafenib, ripretinib), which are not currently available for those patients that progress on avapritinib. In addition, avapritinib is only approved for patients with D842V in Europe, therefore identifying those that have imatinib sensitive mutations is crucial, because many of these patients may receive no treatment at all. By using our paradigm shifting approach and optimizing TKI treatment sequencing for PDGFRA-mutant GIST, treating eligible patients with imatinib instead of avapritinib will transform patient care and clinical outcomes.

(P 26) PHASE 1 STUDY OF IDRX-42 IN PATIENTS WITH ADVANCED GASTROINTESTINAL STROMAL TUMORS RESISTANT TO PRIOR SYSTEMIC THERAPY: EARLY RESULTS

Michael C. Heinrich, MD[1]; Nick Lydon[2]; Jaap Verweij[2]; Sean Kim[2]; Patrick Schöffski, MD, MPH[3]; Jonathan Trent, MD, PhD[4]; César Serrano, MD, PhD[5]; Sebastian Bauer, MD[6]; Margaret von Mehren, MD[7]; George Demetri, MD[8]; Vivek Kadambi, PhD[8]; Jessica K. Christo, MA[8]; Debbie Johnson, BS[8]; James Shao[2]; Suzanne George, MD[9], (1) Portland VA Health Care System and Oregon Health & Science University Knight Cancer Institute, Portland, OR, (2) IDRx, Inc., Plymouth, MA, (3) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium, (4) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, (5) Medical Oncology Department, Hospital Universitario Vall d'Hebron, Vall d'Hebron Institute of Oncology, Barcelona, Spain, Barcelona, Catalonia, Spain, (6) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (7) Fox Chase Cancer Center, Philadelphia, PA, (8) IDRx, Inc, Plymouth, MA, (9) Dana-Farber Cancer Institute, Boston, MA

Objective: IDRX-42 is a potent, highly selective, oral inhibitor of multiple mutant variants of the KIT tyrosine kinase, targeting a range of clinically relevant resistance mutations in KIT as oncogenic drivers of gastrointestinal stromal tumor (GIST). In preclinical studies, IDRX-42 demonstrated superior antitumor activity compared to imatinib in human xenograft models of GIST expressing mutations in KIT exons 9 and 11. In xenograft models expressing secondary resistance mutations in KIT exon 13, IDRX-42 treatment resulted in potent and dose-dependent antitumor activity superior to the second-line standard of care agent, sunitinib (De Sutter 2023). Similar results were seen in a xenograft model expressing secondary resistance mutations in KIT exon 17. We present preliminary results of the ongoing phase 1 first-in-human study evaluating IDRX-42 in patients (pts) with metastatic, surgically unresectable GIST after failure of imatinib or other approved drugs.
Methods: This open-label, phase 1/1b study evaluates the safety, tolerability, pharmacokinetics, and preliminary antitumor activity of orally administered IDRX-42 in adult pts with GIST and documented KIT mutations. The dose escalation phase focuses on establishing the maximum tolerated dose (MTD) of IDRX-42. In the planned phase 1b part of the study, three exploratory cohorts of pts progressing on a defined number of prior lines of systemic therapies at the selected recommended phase 1b dose(s) will be studied to assess antitumor effects and further characterize the safety profile of IDRX-42. The phase 1b portion of the trial will include a second line cohort.
Results: As of October 5, 2023, 33 pts have received IDRX-42 in the ongoing dose escalation

portion of the study. Median age was 60 years, median number of lines of prior systemic therapy was 4, 70% of pts had any KIT exon 11 mutation, and 33% of patients had any KIT exon 9 mutation. With the starting dose of 120 mg QD, 4 dose levels (120, 240, 400 and 600 mg QD) were cleared by the Safety Review Committee and a 400 mg BID cohort is currently under evaluation. The majority of treatment-related adverse events were CTCAE version 5.0 grade 1, the most common (>25%) being diarrhea (70%), nausea (52%), and vomiting (27%). Two events were graded as dose limiting toxicities, one at 600 mg QD (syncope) and one at 400 mg BID (vomiting); both pts chose to proceed with dose reduction and continue on IDRX-42 for more than 5 and 2 months, respectively. Twenty-eight of 33 pts were evaluable for objective response (completed ≥1 response assessment). MTD has not been reached. The disease control rate (partial response [PR] or stable disease ≥ 16 weeks) to date is 67.9% (19 of 28 evaluable pts). Four pts exhibited confirmed PRs per modified RECIST (mRECIST): 1 pt at 120 mg, 1 pt at 400 mg, and 2 pts at the 600 mg dose levels. Tumor shrinkage and confirmed PRs were observed across all KIT mutants treated, including both primary driver and resistance mutations (exons 9, 11, 13, and 17), consistent with the potent multi-mutant KIT inhibitory profile of IDRX-42 (Figure 1). FDG-PET scans indicated early metabolic responses in GIST with IDRX-42, and 23 pts (70%) remain on IDRX-42 with 10 pts discontinued due to GIST progression after a median follow-up of 4 months. No pts discontinued for toxicity. Longitudinal analyses of ctDNA show reductions across all mutation variants in all KIT exons identified. IDRX-42 demonstrated a dose-dependent increase in plasma exposure with a long half-life (~125 hours) and a 5-8 fold accumulation at steady state.

Conclusion: IDRX-42 demonstrates promising antitumor activity and a favorable safety profile in a pre-treated group of pts with advanced/metastatic GISTs. IDRX-42 has a broad and potent KIT inhibitory profile including primary driver and resistance mutations. Phase 1b will evaluate IDRX-42 in pts after failure of first-line imatinib as well as after failure of later lines of therapy.

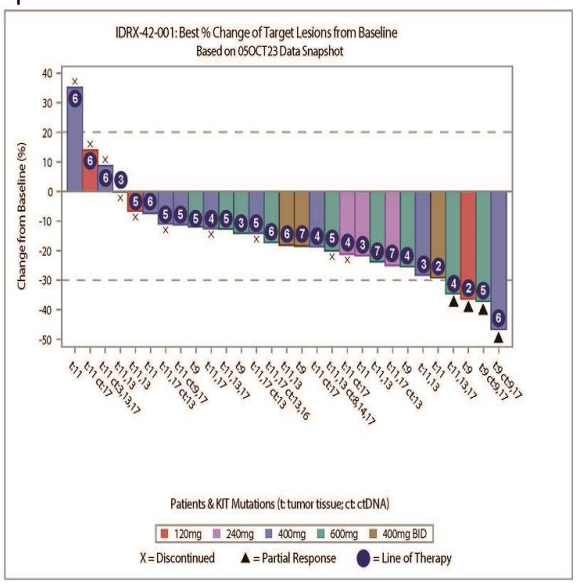


Figure 1:

(P 27) A MULTICENTER RETROSPECTIVE STUDY ON THE CLINICAL BEHAVIOR OF SDH-DEFICIENT GIST

Evelyne Roets, MD[1]; Huma Q. Rana, MD[2]; Suzanne George, MD[2]; Ramesh Bulusu, MD, MRCP,MSc,FRCR[3]; Robin L. Jones, BSc, MB BS, MRCP, MD[4]; Elena Fumagalli, MD[5]; Bruno Vincenzi, MD, PhD[6]; Silvia Gasperoni, MD[7]; Jean-Yves Blay, MD, PhD[8]; Maria Pantaleo, MD PhD[9]; Margherita Nannini, MD[10]; Leonidas Apostolidis, MD[11]; Georg Martin Haag, MD[12]; Javier Martin-Broto, MD, PhD[13]; David S. Moura, PhD[14]; César Serrano, MD, PhD[15]; Barbara Denecke[16]; Hans Prenen[17]; Daria Filonenko, MD PhD[18]; An Reyners, Prof. Dr.[19]; Astrid W. Oosten, MD PhD[20]; Hans Gelderblom, MD, PhD[21]; Ingrid M. Desar, MD PhD[22]; Winette van der Graaf, Prof. Dr.[23]; Winan J. van Houdt, MD, PhD, MS[23]; Neeltje Steeghs, Dr.[23]; Sameer Rastogi, DM[24]; Alexander Guminski, MD/PhD[25], (1) Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands, (2) Dana-Farber Cancer Institute, Boston, MA, (3) Department of Medical Oncology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, England, United Kingdom, (4) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (5) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (6) Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Lazio, Italy, (7) AOU Careggi, Firenze, Toscana, Italy, (8) Centre Léon-Bérard, Lyon, Rhone-Alpes, France, (9) Institute of Hematology and Medical Oncology L & A Seragnoli and Interdepartmental Centre for Cancer Research G Prodi, Bologna, Emilia-Romagna, Italy, (10) IRCSS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Emilia-Romagna, Italy, (11) National Center for Tumor Diseases (NCT) Heidelberg, Heidelberg, Baden-Wurttemberg, Germany, (12) National Center for Tumor Diseases (NCT) Heidelberg, Heidelberg University Hospital, Heidelberg, Baden-Wurttemberg, Germany, (13) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain, (14) Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM),, Madrid, Madrid, Spain, (15) Medical Oncology Department, Hospital Universitario Vall d'Hebron, Vall d'Hebron Institute of Oncology, Barcelona, Spain, Barcelona, Catalonia, Spain, (16) Kantonsspital St. Gallen, Sankt Gallen, Sankt Gallen, Switzerland, (17) University Hospital Antwerp, Antwerpen, Belgium, (18) SBIH Moscow Clinical Scientific and Practical Center Named After A.S.Loginov of DHM Moscow, Moscow, Moskva, Russia, (19) University medical center Groningen, Groningen, Groningen, Netherlands, (20) Erasmus MC Cancer Institute, Rotterdam, Zuid-Holland, Netherlands, (21) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (22) Radboud University Medical Center, Nijmegen, Gelderland, Netherlands, (23) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (24) Department of medical oncology, Dr BRAIRCH, All India Institute of Medical Sciences, New Delhi, Delhi, India, (25) Royal North Shore Hospital, St Leonards, New South Wales, Australia

Objective: Three percent of gastrointestinal stromal tumors (GIST) show succinate dehydrogenase (SDH) deficiency on immunohistochemistry (IHC) which is caused either by a germline mutation in one of the SDH genes, or by SDHC promotor hypermethylation. Little is known about SDH-deficient GIST but some studies suggest that the clinical presentation of SDH-deficient GIST is different from KIT/PDGFRA mutated GIST. The aim of this study is to investigate the patient characteristics and clinical behavior of SDH-deficient GIST.

Methods: In this international, multicenter, retrospective study patients with IHC proven SDH-deficient GIST with or without SDH germline mutations were included. Patient and tumor

characteristics, treatment and SDH-related variables were collected. Follow-up time was defined as the interval between GIST diagnosis and the moment of data collection or death.

Results: We included 104 patients treated in 16 institutions worldwide. Of the 79 (76%) patients with germline mutations 39 (49%) were in SDHA, 25 (32%) in SDHB, 13 (16%) in SDHC and two (3%) were in the SDHD gene. In the remaining patients: no SDH germline mutation was identified (n=6), germline mutation testing was not performed (n=11), SDHC promotor hypermethylation was found (n=2), results were pending (n=2) or missing (n=1), or a variant of unknown significance (n=3) was found. The majority of patients were female (65/104, 63%) and the median age at diagnosis was 35 (range 9-77) years. Of the 94 patients (90%) with a gastric GIST, 23 patients (25%) had multifocal gastric GIST. The stage at diagnosis was localized, metastatic or unknown in 54 (52%), 40 (38%) and 10 patients (10%) respectively. The most common metastatic locations were liver (43/104, 41%) and peritoneum (23/104, 22%). Carney-Stratakis syndrome and Carney triad were reported in 18 (17%) and 6 (6%) patients, respectively. In most patients treatment consisted of surgery alone (46/104, 44%) or surgery combined with systemic treatment (40/104, 38%). The most commonly used systemic therapies were imatinib (44/104, 42%), sunitinib (32/104, 31%) and regorafenib (21/104, 20%). During the median follow-up time of 11 years, (range < 1-40 years), 19 patients treated for localized disease (19/54, 35%) developed a recurrence (median time to recurrence 4, range < 1-23 years). Six patients (6/54, 11%) developed a recurrence ≥10 years after diagnosis. GIST was reported as the cause of death in 9 patients and for 5 patients the cause of death was not reported.

Conclusion: The clinical behavior of SDH-deficient GIST is different from KIT/PDGFRA mutated GIST. While GISTs normally present later in life, SDH-deficient GISTs occur in much younger patients and show a strong gastric predilection. We observed a high frequency of metastatic disease and a high recurrence rate, including late recurrences. Despite the ineffectiveness of imatinib in SDH-deficient GIST, almost half of patients received imatinib. However, imatinib might have been started before IHC and mutation analysis were performed and the duration of treatment was not reported. These results show that SDH-deficient GISTs require a different diagnostic and treatment approach.

Table 1 Characteristics of patients with SDH-deficient GIST (total cohort)

	All patients, n=104 (%)
Sex	
Male	39 (38)
Female	65 (63)
Median age at GIST diagnosis in years[a]	35 (9-77)
SDH germline mutation	
Yes	79 (76)
No	6 (6)
SDHC promotor hypermethylation	2 (2)
Not tested for germline mutation	11 (11)
Germline testing pending	2 (2)
Variant of unknown significance	3 (3)
Missing	1 (1)
GIST location	
Gastric	94 (90)
Unknown	3 (3)
Other[b]	7 (7)
Multifocal GIST	
Yes	23 (22)
No	75 (72)
Unknown	6 (6)
Stage at diagnosis[c]	
Localized	54 (52)
Metastatic	40 (38)
Unknown	10 (10)
Location of metastasis[d]	
Liver	43 (41)
Peritoneal	23 (22)
Lymph nodes	7 (7)
Adrenal	2 (2)
Bone	2 (2)
Mesenterium	2 (2)
Omentum	3 (3)
Pancreas	4 (4)
Lung	2 (2)
Types of treatment received	
Surgery only	46 (44)
Systemic treatment only	13 (13)
Surgery and systemic treatment[e]	40 (38)
Expectative	3 (3)
Unknown	2 (2)
Total gastrectomy	
Yes	12 (12)
No	90 (87)
Missing	2 (2)
Type of systemic therapy	
Imatinib	44 (42)
Sunitinib	32 (31)
Regorafenib	21 (20)
Pazopanib	6 (6)
Other[f]	10 (10)
Recurrence	
Yes	19 (35)
No	35 (65)
Not applicable[g]	50

Table 1 Characteristics of patients with SDH-deficient GIST (total cohort)

Median duration of follow-up in years[h]	11 (<1-40)
Median time to recurrence in years	4 (<1-23)
Other tumors	
Carney-Stratakis syndrome[i]	18 (17)
Carney triad[j]	6 (6)
Colorectal cancer	1 (1)
Renal cell carcinoma	1 (1)
Breast cancer	2 (2)
Esophageal leiomyoma	1 (1)
Melanoma	3 (3)
Thyroid cancer	2 (2)
Desmoid fibromatosis	1 (1)
Sarcoma[k]	2 (2)
Pituitary tumor	1 (1)
Testicular cancer	1 (1)
Status at last follow-up	
Alive	88 (85)
Died due to GIST	9 (9)
Reason of death not reported	5 (5)
Lost to follow-up	2 (2)

GIST, gastrointestinal stromal tumor; SDH, succinate dehydrogenase.

[a] For one patient age at GIST diagnosis was unknown.

[b] Small bowel (n=2), duodenum (n=1), attached to ileum (n=1), gastroesophageal junction (n=1), omentum (n=1), abdomen not specified (n=1).

[c] Gastric GISTs with lymph node involvement were classified as 'metastatic'.

[d] Location of metastasis in patients with metastatic disease at diagnosis or metastatic recurrence. As patients can have multiple locations of metastasis the cumulative percentage does not add up to 100.

[e] Five of these patients were also treated with (selective internal) radiotherapy, radiofrequency ablation or embolization.

[f] Sorafenib (n=2), temozolomide (n=1), nilotinib (n=3), binimetinib (n=1), hsp90 inhibitor (n=1), regoratinib (n=1), epirubicin and ifosfamide (n=1).

[g] Not applicable, i.e. patients with metastatic disease at diagnosis or unknown stage at diagnosis.

[h] Median follow-up time reflects the time from GIST diagnosis until death or censoring.

[i] The combination of gastric GIST and paraganglioma.

[j] The combination of gastric GIST, pulmonary chondroma, and paraganglioma. Two patients had an incomplete Carney triad with only GIST and pulmonary chondroma.

[k] The type of sarcoma was not reported.

(P 28) RETROSPECTIVE ANALYSIS OF SUCCINATE DEHYDROGENASE (SDH)-DEFICIENT GASTROINTESTINAL STROMAL TUMOR (GIST) OUTCOMES: AN INTERNATIONAL STUDY OF 256 PATIENTS

Ashwyn K. Sharma, M.D.[1]; Yuwei Cheng, B.A.[2]; Tahsin M. Khan, M.D.[3]; Emily Verbus, M.D.[3]; Alexander Rossi, M.D.[3]; Adam M. Burgoyne, MD, PhD[4]; Paul T. Fanta, MD[5]; Denisse A. Evans, MSc[6]; Mark Antkowiak, M.D.[1]; Barbara J. Thomas, RN, BSN[7]; Jill P. Mesirov, PhD[8]; Jeremy L. Davis, MD[3]; Sara Rothschild, M.P.H.[6]; John Glod, MD, PhD[9]; Lin Liu, Ph.D.[2]; Andrew M. Blakely, MD[10]; Jason K. Sicklick, M.D., FACS[1], (1) Department of Surgery, Division of Surgical Oncology, University of California San Diego, San Diego, CA, (2) Division of Biostatistics and Bioinformatics, Herbert Wertheim School of Public Health and Human Longevity Science, University of California San Diego, San Diego, CA, (3) Surgical Oncology Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, (4) Department of Medicine, Division of Hematology-Oncology, UC San Diego Health, La Jolla, CA, (5) Department of Medicine, Division of Medical Oncology, University of California San Diego, La Jolla, CA, (6) The Life Raft Group, Wayne, NJ, (7) Center for Cancer Research, National Cancer Institute, Bethesda, MD, (8) Department of Medicine, Division of Genomics and Precision Medicine, University of California San Diego, La Jolla, CA, (9) Center for Cancer Research/National Cancer Institute, Bethesda, MD, (10) Surgical Oncology Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, Bethesda, MD

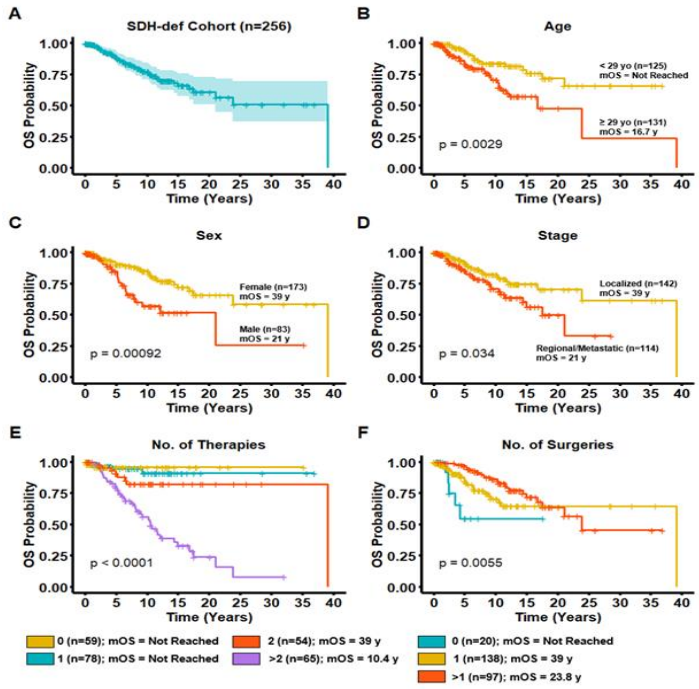
Objective: About 5-10% of GIST are caused by germline mutations in the SDH genes or epigenetic silencing of the SDHC promoter. Both mechanisms lead to loss of a functional SDH complex and therefore SDH-deficiency. Limited data on these tumors from case reports/series often describe SDH-deficient (SDH-def) GIST as "indolent," pediatric, insensitive to tyrosine kinase inhibitors, and recalcitrant to serial or more extensive surgical resections. Yet, we are seeing SDH-def GIST patients dying from this cancer with limited understanding of why their disease is more aggressive. Therefore, we sought to combine data from an SDH-def GIST referral center (UCSD), the NIH GIST Clinic and the Life Raft Group (international patient advocacy group with a patient-reported database) to examine this understudied population.

Methods: Patients with pathologically diagnosed SDH-def GIST before April 30, 2021 were identified. Duplicate patients were counted once. We then examined demographic, clinicopathologic, and mutation profiles, as well as clinical management, and prognostic factors of overall survival (OS). We used Kaplan-Meier analyses and a multivariable Cox Proportional Hazard model to identify important clinical factors. The final model was determined via backwards selection and was stratified based on covariates that violated the proportional hazards ratio.

Results: Overall, 256 SDH-def GIST patients were included. The median age at diagnosis was 29 years (range: 5-79) with 68% females. Of these patients, 55.5% presented with localized disease vs. regionally advanced (11.7%) or metastatic (32.8%) disease. The most common mutations were SDHA (18.4%) and SDHB (17.6%), though many patients had unknown SDH mutations (49.6%), likely reflecting lack of access to mutational testing. For all patients, the median OS was 39 years after diagnosis (Fig. A) with an 88.8% and 77.4% OS rate at 5- and 10-years, respectively. We then performed univariate OS analyses (Fig. B-F). Patients who were age ≥29 years at diagnosis [HR 2.36, 95% CI 1.32-4.21 p=0.004], male [HR 2.51, 95% CI 1.43-4.42, p=0.001], or had regionally advanced or metastatic disease at diagnosis [HR 1.81, 95% CI 1.04-3.16, p=0.034 compared to localized] had worsened OS rates. Furthermore,

patients treated with sunitinib [HR 7.25, 95% CI 3.26-16.10, p< 0.001], regorafenib [HR 3.27, 95% CI 1.79-5.97, p< 0.001], or temozolomide [HR 3.58, 95% CI 1.40-9.16, p=0.008] had worsened OS. These likely represent surrogates for more aggressive biology or extensive disease. In fact, receipt of >2 drug therapies was associated with worse prognosis [HR 8.77, CI 3.13-24.60, p< 0.001] compared to those with only 1 drug therapy. But, contrary to an earlier study, surgical resection was associated with a decreased hazard of death and improved OS [HR 0.279, 95% CI 0.11-0.72, p=0.008]. On multivariable analysis, backwards selection resulted in age, sex, sunitinib, number of lines of therapy, and number of surgeries as significant covariates. Due to differences in underlying hazards, the models were stratified based on sunitinib use and number of surgeries. In the final model, older age at diagnosis, male sex, and number of therapies were all associated with worsened OS (Table).

Conclusion: To our knowledge, this represents the largest SDH-def GIST analysis to date. Despite being believed to be mostly confined to the pediatric and adolescent population, and still often mislabeled as Pediatric GIST, SDH-def GIST occurs across the age spectrum, and a diagnosis later in life portends a worse prognosis. Moreover, male sex is a poor prognostic factor, raising questions as to the underlying biological differences to explain this finding. For the first time, we also show that surgical resection is associated with improved survival in this patient population. Finally, in contrast to other GIST subtypes, advanced stage at diagnosis does not independently correlate with OS, suggesting that further studies are needed to develop prognostic models.



Variable		Multivariate HR (95% CI)			P-value
		HR	2.50%	97.50%	
Age (years, continuous)		1.019	1.000	1.038	0.05
Sex	Female	Reference	.	.	.
	Male	2.292	1.278	4.111	0.005
No. of Therapies (continuous)		1.192	1.023	1.388	0.024

(P 29) COMBINATION TREATMENT WITH TEMOZOLOMIDE AND THE DEATH RECEPTOR-5 AGONIST INBRX-109 EFFECTIVELY TARGETS SUCCINATE DEHYDROGENASE -DEFICIENT GASTROINTESTINAL STROMAL TUMOR

Shashwath Kalladi Malli, PhD[1]; Ashwyn K. Sharma, M.D.[1]; Manikandan Murugesan, PhD[1]; Kentson Lam, MD, PhD[2]; Chloe Miller, Undergraduate[3]; Diwakar Guragain, PhD[1]; Chih-Min Tang, Ph.D.[1]; Adrian Sanchez-Fdez, PhD[1]; Terrence M. Doherty, B.A.[2]; Paul T. Fanta, MD[4]; Adam M. Burgoyne, MD, PhD[5]; Robert A.J. Signer, Ph.D.[3]; Katelyn M. Willis, PhD[6]; Vasily Andrianov, MD[6]; Jill P. Mesirov, PhD[7]; Brendan Eckelman, PhD[6]; Jason K. Sicklick, M.D., FACS[1], (1) Department of Surgery, Division of Surgical Oncology, University of California San Diego, San Diego, CA, (2) Moores Cancer Center, University of California San Diego, San Diego, CA, (3) Moores Cancer Center, University of California, San Diego, San Diego, CA, (4) Department of Medicine, Division of Medical Oncology, University of California San Diego, La Jolla, CA, (5) Department of Medicine, Division of Hematology-Oncology, UC San Diego Health, La Jolla, CA, (6) Inhibrx, Inc, La Jolla, CA, (7) Department of Medicine, Division of Genomics and Precision Medicine, University of California San Diego, La Jolla, CA

Objective: Succinate Dehydrogenase (SDH)-def Gastrointestinal Stromal Tumor (GIST) may present as indolent tumors or aggressive cancers that respond poorly to current FDA-approved therapies for GIST. They are caused by inherited mutations in the SDH (A/B/C/D) genes, or by epigenetic silencing of the SDHC promoter. Both causes result in loss of a functional SDH complex that abolishes the cell's ability to convert succinate to fumarate. In turn, elevated succinate levels suppress the homologous recombination DNA Damage Repair (DDR) pathway required to resolve DNA double-strand breaks, thus rendering SDH-def tumor cells vulnerable to temozolomide (TMZ). Furthermore, we have shown that TMZ induces endoplasmic reticulum

(ER) stress, which causes SDH-def GIST to upregulate DR5 (Death Receptor 5) expression. We now hypothesize that DR5 is a therapeutic vulnerability induced by TMZ, and that combination treatment with INBRX-109, a tetravalent DR5 agonist, will enhance the efficacy of TMZ monotherapy

Methods: We previously reported the development of the 3 human SDH-def cell models isolated from patients with SDH-def GIST (Yebra et al., CCR 2022). Using these models, we performed immunoblot analyses, single cell (sc) RNAseq, flow cytometry-based ER stress assays, and pharmacologic synergy analyses with 4 methods (Bliss, ZIP, Loewe, HSA). We also performed single nucleus (sn) RNAseq in 13 SDH-def GIST. Finally, we performed DR5 immunohistochemical (IHC) staining of resected SDH-def GIST treated with TMZ in a clinical trial (NCT03556384).

Results: Using the 3 cell models, immunoblotting revealed that TMZ treatment significantly increased expression of the transcription factor CHOP, which in turn increased DR5 expression. snRNAseq of 13 SDH-def GIST also showed that multiple TMZ-naïve tumor cell clusters had basal DR5 expression. In vitro, we utilized scRNAseq to confirm that SDHC mutant GIST cells treated with TMZ (600 μM, 7d) had increased DR5 expression versus untreated (control) cells in most cell clusters. Finally, IHC staining of 3 TMZ-treated SDH-def GIST showed high DR5 immunostaining, confirming our in vitro findings. We then treated the SDHA and SDHC mutant cell models with TMZ+INBRX-109 and performed cell viability assays. These showed that combination treatment is either additive (0-9.9) or synergistic (≥10) in targeting cells in vitro. To further understand the mechanism of cell killing, we performed flow cytometric quantitation analyses of polyubiquitination and tetraphenylethene maleimide (TMI), which independently confirmed that TMZ+INBRX-109 increased global ER stress in cells. Further analyses showed significantly increased levels of both mRNA (scRNAseq) and protein (immunoblot) expression of PERK (protein kinase R-like ER kinase) pathway components in ER stress signaling (i.e., p-eIF2α, ATF4, CHOP, DR5) and apoptotic pathways (i.e., BAX, CASP3). Hence, treatment with TMZ+INBRX-109 activated two independent mechanisms that each led to tumor cell death, namely: 1) the DDR pathway; and 2) the PERK ER stress pathway leading to increased apoptosis. Finally, in an unbiased high-throughput drug screen using a chemical library of 5,034 compounds, we identified 4 compounds with IC50< 100 nM in the 3 SDH-def GIST models that were ER stress inducers, further suggesting that this pathway may represent an effective target in SDH-def tumor cells.

Conclusion: Herein, we report that SDH-def tumors are sensitive to TMZ, which induces ER stress and upregulates DR5, a pro-apoptotic receptor that can be targeted by INBRX-109 (Figure 1). In turn, TMZ+INBRX-109 additively/synergistically reduces SDH-def GIST cell viability in vitro. These data provide the preclinical evidence for a Phase I clinical trial (3+3 dose escalation design with two dosing cohorts, NCT03715933) to study the safety/tolerability of TMZ+INBRX-109 in patients with aggressive SDH-def cancers, including GIST (Figure 2). As of the submission date, the first subject has been safely dosed and a second subject is in screening.

Figure 1. TMZ and INBRX-109 collaborate to induce ER stress and apoptosis in SDH-def GIST cells.

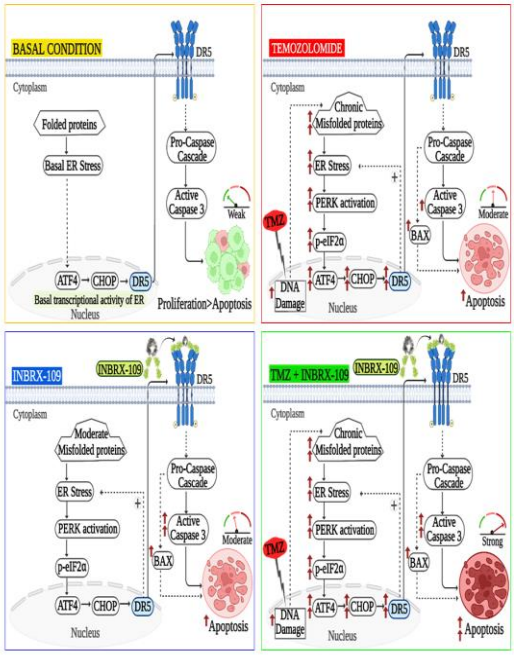


Figure 2. Schema of the phase 1 study on INBRX-109 in subjects with locally advanced or metastatic solid tumors including sarcomas (NCT03715933)

C5: SDH-def solid tumors or GIST			
Cohort	**Status**	**INBRX-109 Dosing**	**TMZ Dosing**
C5a n=3-4	**Open**	**1 mg/kg** C1D1 → 3 mg/kg C2D1 or beyond*; IV every 4 weeks (Q28D)	Temozolomide 85 mg/m2/day PO on Day 1 through Day 21 of 28 day cycle (3Wk on, 1Wk off)
C5b n=3-4	**n/a**	**3 mg/kg**; IV Q28D	
C5c n=12-14	**n/a**	RP2D (e.g., **1 mg/kg or 3 mg/kg**) IV Q28D	

*→escalation after safety review
** → After 6 cycles, irinotecan and/or temozolomide may be stopped or continued per investigator preference. INBRX-109 to be continued.

(P 30) FINANCIAL DIFFICULTIES EXPERIENCED BY DUTCH GASTROINTESTINAL STROMAL TUMOR (GIST) PATIENTS: DATA FROM A CROSS-SECTIONAL MULTICENTER STUDY

Deborah van de Wal, MD[1]; Dide den Hollander, MD, PhD[2]; Ingrid M. Desar, MD PhD[2]; Hans Gelderblom, MD, PhD[3]; Astrid W. Oosten, MD PhD[4]; An Reyners, Prof. Dr.[5]; Neeltje Steeghs, Dr.[1]; Winette van der Graaf, Prof. Dr.[1]; Olga Husson, PhD[6], (1) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (2) Radboud University Medical Center, Nijmegen, Gelderland, Netherlands, (3) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (4) Erasmus MC Cancer Institute, Rotterdam, Zuid-Holland, Netherlands, (5) University medical center Groningen, Groningen, Groningen, Netherlands, (6) Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands

Objective: Financial difficulties get little attention in daily clinical practice, while they can have a considerable impact on treatment outcomes and the patient's quality of life. This study aims to explore the prevalence of financial difficulties among GIST patients, with a distinction in patients on tyrosine kinase inhibitor (TKI) treatment or not on TKI treatment, and investigate associations between financial difficulties and sociodemographic and clinical characteristics, work, cancer-related concerns, symptoms of anxiety and depression.

Methods: A cross-sectional study was conducted among Dutch GIST patients between September 2020 and June 2021. Patients completed nine items regarding financial difficulties of the EORTC item bank, work-related questions, Hospital Anxiety and Depression Scale (HADS), Cancer Worry Scale (CWS) and EORTC QLQ-C30.

Results: In total, 328 GIST patients participated (response rate 63.0%), of which 108 (32.9%) were on TKI treatment. Experiencing financial difficulties was associated with significantly lower global quality of life (67.8 vs 81.6, p=< 0.001) as compared to patients who did not report financial difficulties. Patients with financial difficulties also more frequently reported symptoms of anxiety (41.7% vs 11.5%, p=< 0.001) and depression (36.1% vs 10.1%, p=< 0.001), had more severe fear of cancer recurrence or progression (73.0% vs 39.9%, p=< 0.001), and were more often concerned about dying from GIST both in the near future (64.9% vs 34.9%, p=< 0.001) and in the long run (75.7% vs 49.5%, p=0.003). The odds of experiencing financial difficulties was 16.4 (95% CI 1.24-217.1; p=0.03) times higher in patients that were less able to work. Patients currently treated with TKIs experienced significantly more financial difficulties compared to patients not on TKIs (17.6% vs 8.7%, p=0.02).

Conclusion: Even in a country where the costs of TKIs and follow-up care are covered by health insurance, financial difficulties can be present in GIST patients, especially in patients on TKI treatment. Given the association between financial difficulties with quality of life, anxiety, depression and cancer-related concerns, more support should be provided to patients.

(P 31) NEOADJUVANT IMATINIB IN LOCALLY ADVANCED GASTROINTESTINAL STROMAL TUMORS (GIST) IS EFFECTIVE AND SAFE: RESULTS FROM A PROSPECTIVE SINGLE CENTER STUDY WITH 108 PATIENTS

Stijn JC Van der burg, MD[1]; Deborah van de Wal, MD[1]; Evelyne Roets, MD[2]; Neeltje Steeghs, Dr.[1]; Johanna van Sandick, Dr.[1]; Martijn Kerst, Dr.[1]; Frits Van Coevorden, MD PhD[1]; Koen Hartemink, Dr.[1]; Xander Veenhof, Dr.[1]; Anne Miek Koenen, n/a[1]; Yvonne Schrage, MD, PhD[1]; Winan J. van Houdt, MD, PhD, MS[1]; Nikki Ijzerman, Dr.[1]; Winette van der Graaf, Prof. Dr.[1], (1) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (2) Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands

Objective: Neoadjuvant imatinib is considered for gastrointestinal stromal tumors (GIST) when decreased tumor size provides less extensive surgery and higher R0 resection rates. This study evaluates the effectivity and safety of neoadjuvant imatinib for large or locally advanced GIST.

Methods: From the prospective database of the Dutch GIST Consortium, all patients who underwent surgery after neoadjuvant imatinib at our center between 2009 and 2022 were selected. Independent and blinded assessment of surgical strategy was performed by two surgeons, based on anonymized CT-scans before and after neoadjuvant imatinib.

Results: Of 113 patients that received neoadjuvant imatinib, 108 (95%) (mean age 61.6 (SD 11.5), 54% male) underwent a GIST resection. Of all GIST, 67% was localized in the stomach and in 25% in the duodenum or small intestine. In 74% of the GIST, a KIT exon 11 mutation was found. Decreased tumor size was seen in 95 (88%). Having a KIT exon 11 mutation (OR=5.64 [CI 95% 1.67–19.1], p< 0.01) or not having a mutation (OR=0.19 [CI95% 0.04–0.89], p=0.04) were positive and negative predictive values for partial response, respectively. In 55 (51%) patients, there was de-escalation of surgical strategy after neoadjuvant imatinib. Surgical complications were documented in 16 (15%) patients (n=8 grade II, n=5 grade IIIa, n=3 grade IIIb) and R0 resection was accomplished in 95 (89%) patients. The 5-year disease free- and overall survival were 80% and 91%, respectively.

Conclusion: This study shows that neoadjuvant imatinib is effective and safe for patients with large or locally advanced GIST.

Table 1: patient characteristics

	N= 108	
Age (Mean; SD)	61.6	(11.5)
Gender (male; %)	58	(53.7)
BMI (Median; IQR)	25.8	(24.0 – 29.5)
Missing (%)	2	(1.8)
ASA score (%)		
1	41	(38.0)
2	53	(49.1)
3	8	(7.4)
4	1	(0.9)
Missing	5	(4.6)
Mutation		
KIT exon 9	6	(5.6)
KIT exon 11	80	(74.1)
KIT exon 13	2	(1.9)
PDGFR 18 (D842V)	5	(4.7)
PDGFR 18 (other)	5	(4.7)
No mutation	7	(6.5)
Missing	3	(2.8)
Tumor location (%)		
Stomach	72	(66.7)
Small bowel	18	(16.7)
Duodenum	9	(8.3)
Rectum	7	(6.5)
Esophagus	2	(1.9)
Tumor size before imatinib		
<5 cm	9	(8.3)
5-10 cm	46	(42.6)
>10 cm	53	(49.1)
Tumor size after imatinib		
<5 cm	35	(32.4)
5-10 cm	45	(41.7)
>10 cm	28	(25.9)
Mitotic rate after biopsy ≤5/5 mm² (%)	42	(38.8)
Mitotic rate after biopsy >5/5 mm² (%)	17	(15.7)
Missing	49	(45.4)
Miettinen		
Very low - Low	11	(10.2)
Intermediate - High	71	(65.7)
Missing	26	(24.1)
Number of weeks of imatinib (Median; IQR)	31.0	(24.0 - 42.0)
(temporary) Dosage of imatinib		
<400 mg	8	(7.4)
400 mg	67	(62.0)
>400 mg	33	(30.6)
CTCTAE >2		
3	11	(10.2)
4	1	(0.9)

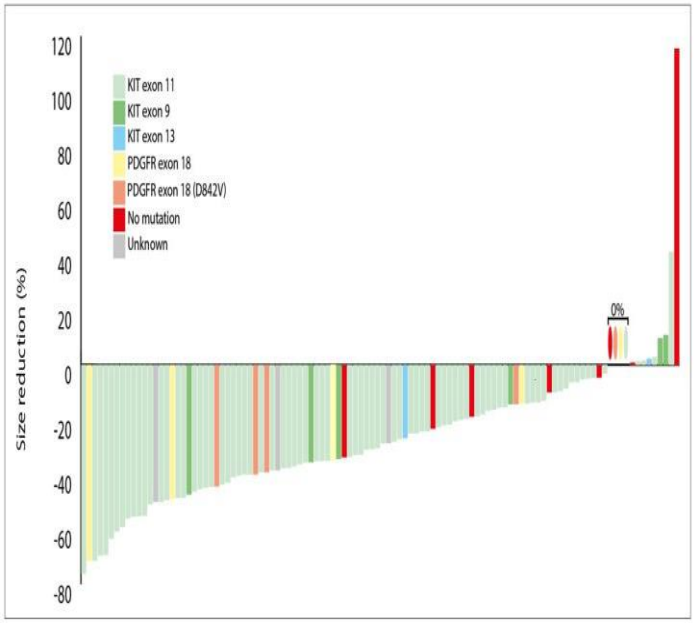


Figure 2: A waterfall plot showing the mutation of all patients together with the percentage of size

(P 32) IMPROVEMENT OF QUALITY AND TEXTBOOK OUTCOMES OF GASTRIC GASTROINTESTINAL STROMAL TUMOR (GIST) RESECTIONS IN THE PAST 13 YEARS; THE INFLUENCE OF MINIMAL INVASIVE SURGERY (MIS)

Stijn JC Van der burg, MD[1]; Roos Bleckman, MD[2]; Stefanie N. Hakkesteegt, MD[3]; Neeltje Steeghs, Dr.[1]; Dirk J. Grünhagen, MD, PhD[3]; An Reyners, Prof. Dr.[2]; Boudewijn Van etten, Dr.[2]; Han Bonenkamp, Dr.[4]; Henk H. Hartgrink, Dr.[5]; Michel Wouters, Prof. Dr.[1]; Winan J. van Houdt, MD, PhD, MS[1]; Yvonne Schrage, MD, PhD[1]; Frits van Coevorden, MD, PhD[6]; Koen Hartemink, Dr.[1]; Johanna van Sandick, Dr.[1], (1) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (2) University medical center Groningen, Groningen, Groningen, Netherlands, (3) Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands, (4) Radboud university medical center, Nijmegen, Gelderland, Netherlands, (5) Leiden university medical center, Leiden, Zuid-Holland, Netherlands, (6) Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands

Objective: The Dutch gastrointestinal stromal tumor (GIST) consortium, comprising five GIST expert centers, established the prospective Dutch GIST Registry (DGR) in 2009 to facilitate research and to enhance the quality of GIST care. Changes in the quality of resections of primary localized gastric GIST has not been described so far. This study aims to describe the differences observed over time for this patient population in indications for surgical resections and surgical outcomes. GIST-specific textbook outcomes (TO) were used as composite measure for surgical outcomes.

Methods: All patients with primary surgically treated localized gastric GISTs recorded in the DGR between January 2009 and January 2022 were included in this study. Metastasized disease at time of surgery was an exclusion criteria. Patient and tumor characteristics were analyzed for all included cases. The analysis of surgical outcomes focused on patients of which the resection was performed in an expert center. To evaluate the variation in quality over time, patients were segregated into four equal periods. TO was defined as clear margins, no perioperative tumor rupture, no surgical complication (Clavien Dindo ≥2), no resurgery or readmission within 90 days after resection, and no prolonged hospital stay (>75th percentile). Multivariate regression analyses were done to search for factors influencing the perioperative outcomes.

Results: A total of 472 patients were included in the study. Patient and tumor characteristics remained consistent over time, except for the median age (62 vs. 65 vs. 68 vs. 68, $p = 0.002$) and the proportion of minimal invasive surgery (MIS) per period (7% vs. 17% vs. 42% vs. 60%, $p < 0.001$). Of the included patients, 385 (81.6%) underwent surgery in an expert center. The proportion of achieved TO increased significantly over time (54% vs. 53% vs. 66% vs. 76%, $p < 0.001$). Multivariate regression analyses showed that achieving TO was significantly correlated with a later period of surgery (OR 1.68 [CI 95% 1.28-2.23], $p < 0.001$), lower age (OR 0.97 [CI 95% 0.95-0.99], $p = 0.004$), MIS (OR 0.97 [CI 95% 0.95-0.99], $p = 0.004$), and lower ASA scores (OR 0.65 [CI 95% 0.44-0.97], $p = 0.035$). Furthermore, a decrease was seen in the median duration of surgery in minutes (92 vs. 94 vs. 77 vs. 73, $p=0.007$) and the median length of hospital stay following surgery in days (6 vs. 6 vs. 5 vs. 4, $p< 0.001$).

Conclusion: The quality of perioperative outcomes for resections of gastric GIST in the five Dutch expert centers has improved over time. Furthermore, its efficiency has increased leading to a decreased duration of surgery and hospital stay.

Table 2: perioperative outcomes of the total population treated in expertise centers and that population

	Total		Over time								
			Period 1		Period 2		Period 3		Period 4		
	N= 385		N= 50		N= 112		N= 123		N= 100		p
Irradical resections (%)	24	(6.2)	4	(8.0)	8	(7.1)	6	(4.9)	6	(6.0)	0.840
Missing	1	(0.3)	0	(0.0)	1	(0.9)	0	(0.0)	0	(0.0)	
Preoperative tumor ruptures (%)	12	(3.1)	3	(6.0)	4	(3.6)	2	(1.6)	3	(3.0)	0.499
Missing	2	(0.5)	0	(0.0)	2	(1.8)	0	(0.0)	0	(0.0)	
Postoperative complications (%)	52	(13.5)	11	(22.0)	17	(15.2)	14	(11.4)	10	(10.0)	0.146
Missing	4	(1.0)	2	(4.0)	1	(0.9)	1	(0.8)	0	(0.0)	
Reoperations (%)	10	(2.6)	1	(2.0)	2	(1.8)	4	(3.3)	3	(3.0)	0.891
Missing	4	(1.0)	0	(0.0)	2	(1.8)	1	(0.8)	1	(1.0)	
Readmissions (%)	14	(3.6)	3	(6.0)	3	(2.7)	4	(3.3)	4	(4.0)	0.769
Missing	4	(1.0)	0	(0.0)	2	(1.8)	1	(0.8)	1	(1.0)	
>75% length of hospital stay	103	(26.8)	21	(42.0)	39	(34.8)	32	(26.0)	11	(11.0)	**<0.001**
Missing	6	(1.6)	1	(2.0)	2	(1.8)	1	(0.8)	2	(2.0)	
Textbook outcome (%)	237	(61.6)	27	(54.0)	59	(52.7)	81	(65.9)	76	(76.0)	**<0.001**
Missing	10	(2.6)	2	(4.0)	5	(4.5)	2	(1.6)	1	(1.0)	
Duration of surgery (min) (IQR)	83	(60 - 115)	92	(95 - 147)	94	(62 - 125)	77	(55 - 109)	73	(56 – 94)	**0.007**
Missing (%)	42	(10.9)	3	(6.0)	7	(6.3)	16	(13.0)	16	(16.0)	
Duration of admission in days (IQR)	5	(4 - 7)	6	(5 - 9)	6	(5 - 7)	5	(4 - 7)	4	(3 - 6)	**<0.001**
Missing (%)	6	(1.6)	1	(2.0)	2	(1.8)	1	(0.8)	2	(2.0)	

(P 33) **LONGITUDINAL FOLLOW UP OF GASTROINTESTINAL STROMAL TUMORS:DATA FROM THE LIFE RAFT GROUP INTERANTIONAL PATIENT REGISTRY**

Denisse A. Evans, MSc; Jerry W. Call; Sahibjeet Parmar, MPH; Antoinette Pauwels, MPH; Sara Rothschild, M.P.H., The Life Raft Group, Wayne, NJ

Objective: Gastrointestinal stromal tumors (GISTs) are rare soft tissue sarcoma neoplasms. Due to its rarity and heterogeneity nature, The Life Raft Group (LRG) international GIST Patient Registry conducts a retrospective, longitudinal, natural history study with patient-reported data with the goal of identifying patterns, understanding genomic profiles, and developing real-world evidence-based data. Here, we conduct a comprehensive analysis of the data collected, with the objective of gaining valuable insights into the epidemiology, clinical characteristics, treatment patterns, and long-term outcomes of patients diagnosed with GIST.

Methods: We performed a retrospective analysis of patients with a confirmed GIST diagnosis that enrolled in the LRG patient registry between 2000 to 2022 (N=2465). Demographic data and clinicopathological features, including tumor presentation, tumor size, stage at diagnosis, treatment, and mutational status were analyzed. Overall survival (OS) and Recurrence Free Survival (RFS) were assessed by Kaplan-Meier curve analysis. For all OS and RFS analyses, we excluded all patients diagnosed before the year 2000 to avoid survival bias (n=2267). For RFS analysis, all patients with duration of less than one month were excluded from the analysis.

Results: 2267 patients were included in the study. Of these, the median age at diagnosis was 51 years old [range:5-78]. 53% (n=1202) were females and presented a higher median OS (17.7 years) when compared to males (10.6 years) [HR 1.80, 95% CI 1.55-2.10, p< 0.00001]. 69%(n=1544) of patients had a single tumor upon diagnosis and had a median OS of 17.2

years. Primary tumors occurred in the stomach 47.8% (n=1089), small intestine, 39.7% (n= 904), rectum/anus 3.1% (n=71), colon 2.3% (n=53) and esophagus 0.9% (n=21). When comparing single tumor patients, females had a higher OS than males, 19.5 and 12.8 years respectively. 57.1% (n=1294) of patients received mutational testing and 42.9%(n=973) did not receive testing. Out of the patients that received mutational testing, the mutational distribution was: KIT 81.7% (n= 1058), PDGFRA 8.0% (n= 104), SDHx 7.7% (n= 100), NF1 1.5% (n= 20), BRAF (includes 1 fusion) 0.6% (n= 8), ETV6-NTRK3 fusions 0.2% (n= 2). There was also one case with PTCH1 mutation and one variant of unknown significance in NTRK3 gene. When accounting for mutational status, females still presented a higher median OS (Table1). All patients reported a total of 4537 medical treatments. Median OS for 1st lines to 7th line treatment are presented in Figure 1. Furthermore, Figure 2 demonstrates that among non-metastatic patients, those who received adjuvant treatment (N=784) exhibited a significantly higher median recurrence-free survival (RFS) of 13.1 years, in contrast to patients who did not receive adjuvant treatment (n=639), with a median RFS of 3.5 years [HR 2.04, p< 0.00001].

Conclusion: LRG patient registry enables a deeper comprehension of the disease's natural progression, encompassing treatment records and patient survival data. Further studies investigating the negative prognosis of male patients are needed to identify any factors contributing to poorer outcomes in this specific population. The median RFS time may not always be a reliable indicator to assess the effectiveness of adjuvant treatment. In such situations, it is more accurate to consider the hazard ratio as a superior measure to determine the treatment's benefits. The drastic drop-off rate in survival after patients transition from first-line treatment highlights the importance and urgency of optimizing all factors related to the duration of first-line treatment in order to achieve a better prognosis, emphasizing the need for comprehensive strategies aimed at maximizing treatment selection and efficacy, minimizing treatment interruptions, and ensuring optimal patient outcomes in GIST management. Lastly, the heterogeneity presented in multiple reported mutations underscores the critical significance of mutational testing for patients undergoing oral chemotherapy. By identifying these mutations, healthcare professionals can tailor treatment plans, ensuring patients receive the most effective and personalized therapies.

Table 1. Gender Analysis by Testing Status

	N=	Median OS (years)	p value	Hazard Ratio	95% CI for HR Upper	Lower
Gender						
Female	1202	17.7	<0.00	1.8	1.55	2.1
Male	1065	10.6	001			
Gender & Mutational Testing Status						
Received testing						
Female	714	18.23			16.88	NA
Male	580	12.43			11.44	NA
Did not receive testing			<0.00 001	2.05		
Female	488	12.76			11.14	18.11
Male	485	7.64			7.05	8.98

Figure 1. Median OS by Treatment Line

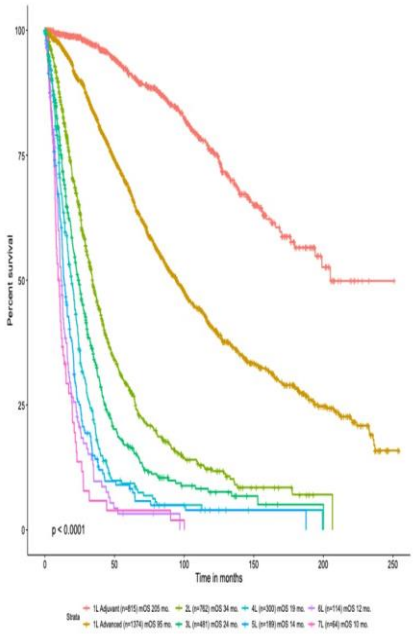
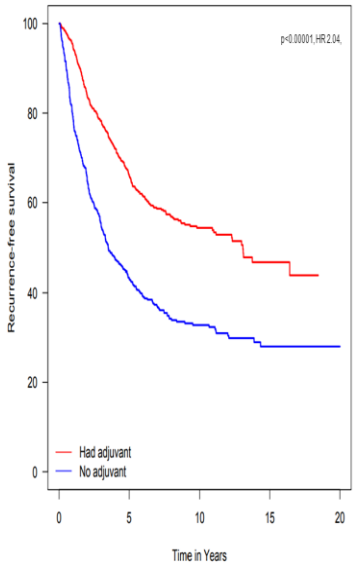


Figure 2. RFS by Adjuvant vs No Adjuvant



(P 34) RIPRETINIB IN COMBINATION WITH OTHER TKIS IN CHINESE PATIENTS WITH ADVANCED GASTROINTESTINAL STROMAL TUMOR: A MULTI-CENTER RETROSPECTIVE ANALYSIS

Lifeng Sun, MD[1]; Yefan Zhang, MD[2]; Xinhua Zhang, MD[3]; JiRen Yu, MD[4]; Jichao Qin, MD[5]; Hailong Qian, MD[6]; Xin Wu, MD[7], (1) The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China (People's Republic), (2) Department of Hepatobiliary Surgery, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, Beijing, China (People's Republic), (3) Department of Gastrointestinal Surgery, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China (People's Republic), (4) The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China (People's Republic), (5) Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hebei, China (People's Republic), (6) Ningbo Medical Center Lihuili Hospital, Ningbo, Zhejiang, China (People's Republic), (7) Chinese PLA General Hospital, Beijing, Beijing, China (People's Republic)

Objective: Although targeted therapies have significantly improved the prognosis for patients with Gastrointestinal stromal tumors (GISTs), resistance to these treatments remains a major challenge. Combining tyrosine kinase inhibitors (TKIs) with other targeted agents may help overcome this resistance in GISTs. When selecting a combination regimen, it is crucial to consider the efficacy and safety of the chosen drug. Ripretinib, a novel switch-control TKI, has demonstrated promising antitumor activity and safety properties in both 2nd and 4th-line treatments of advanced GIST. This is possible to make ripretinib as a preferred backbone in a combination regimen. A previous case report suggested that ripretinib combined with other TKIs could provide clinical benefits for patients with advanced GIST who progressed after

ripretinib treatment. This retrospective analysis aims to further evaluate the effectiveness and tolerability of ripretinib plus other TKIs in patients with advanced GIST from multiple centers.

Methods: Patients diagnosed with GIST, who received ripretinib and sequentially combined it with other TKIs between March 2021 and March 2023, were retrospectively analyzed. Clinicopathological characteristics, treatment regimens, and follow-up visit information were collected. Clinical outcomes, including objective response rate (ORR), progression-free survival (PFS), overall survival, and safety, were assessed.

Results: A total of 10 patients were enrolled, with a median age of 56 years (range, 45-60); 7 (70%) of these patients were female. The data cutoff date was April 20, 2023. The median PFS1 for ripretinib 150 mg QD was 6.8 months (95% CI: 6.05-NA). As the disease progressed, six patients underwent treatment with a combination of Ripretinib and Sunitinib, whereas four patients received Ripretinib in combined with either Regorafenib or Imatinib, with 2 patients for each respective combination (Table 1). Overall, the median PFS2 for combination therapy was 4.7 months (95% CI: 3.15-NA). The best responses to ripretinib combined with other TKIs were PR (10%, 1/10) and SD (60%, 6/10). After a median follow-up of 18.0 months (range: 16.13-NA), the total mPFS and mOS on ripretinib were 13.63 months (95% CI: 9.53-NA) and 18.0 months (95% CI: 17.77-NA), respectively. During the combination therapy period, six patients reported treatment-emergent adverse events (TEAEs), all of which were grade 1/2. The most common TEAEs were palmar-plantar erythrodysesthesia syndrome (30%), diarrhea (20%), and fatigue (20%). A summary of TEAEs is provided in Table 2. No patients experienced serious adverse events (SAEs).

Conclusion: This retrospective study demonstrated meaningful clinical benefits of combining Ripretinib with available TKIs in treating patients with advanced GIST. The safety profile of the combined therapy was considered acceptable. Further research is necessary to establish the optimal dosing regimen for these two targeted drugs and to clarify their mode of action.

(P 35) GENOMIC ALTERATIONS IN ADVANCED GASTROINTESTINAL STROMAL TUMORS AS REVEALED BY COMPREHENSIVE GENOMIC PROFILING TESTS

Hiroyuki Fujii, MD[1]; Hidekazu Hirano, MD, PhD[2]; Kouya Shiraishi, Ph.D.[3]; Hirokazu Shoji, M.D., Ph.D.[4]; Toshiharu Hirose, M.D.[4]; Natsuko Okita, M.D., Ph.D.[4]; Atsuo Takashima, M.D., Ph.D.[4]; Takafumi Koyama, M.D., Ph.D.[5]; Ken Kato, MD, PhD[6], (1) 1) Department of Gastrointestinal Medical Oncology, National Cancer Center Hospital, Tokyo, Japan. 2) Department of Pulmonary Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan., Tsukiji, Tokyo, Japan, (2) Department of Gastrointestinal Medical Oncology, National Cancer Center Hospital, Tokyo, Japan., Chuo-ku, Tokyo, Japan, (3) Division of Genome Biology, National Cancer Center Research Institute, Tokyo, Japan., Tsukiji, Tokyo, Japan, (4) Department of Gastrointestinal Medical Oncology, National Cancer Center Hospital, Tokyo, Japan., Tsukiji, Tokyo, Japan, (5) Department of Experimental Therapeutics, National Cancer Center Hospital, Tokyo, Japan., Tsukiji, Tokyo, Japan, (6) Department of Gastrointestinal Medical Oncology, National Cancer Center Hospital, Tokyo, Japan., Tokyo, Tokyo, Japan

Objective: Gastrointestinal stromal tumors (GISTs) are a rare and aggressive type of mesenchymal tumor that develops in the gastrointestinal tract with heterogeneous molecular features. According to the mutation status of KIT and/or PDGFRA, GISTs are classified into

KIT/PDGFRA-mutated GISTs and wild-type GISTs. Few studies have focused on genomic alterations (GAs) and their applicability for molecular-targeting agents based on comprehensive genomic profiling (CGP) tests for advanced GISTs in clinical practice. We investigated actionable GAs and targetable GAs in advanced GISTs.

Methods: We retrospectively reviewed data on patients with advanced GISTs who underwent CGP tests and were registered with the Center for Cancer Genomics and Advanced Therapeutics (C-CAT) in Japan. The data cutoff was April 2023 using the C-CAT database's latest version 6.0.0. CGP tests were conducted by NCC Oncopanel System (NOP) or FoundationOne CDx (F1). We examined somatic GAs, microsatellite status, and tumor mutation burden (TMB) of KIT/PDGFRA-mutated GISTs (the KIT/PDGFRA group) and wild-type GISTs (the wild-type group). Actionable GAs were counted if they were classified as “pathogenic/oncogenic” or “likely pathogenic/likely oncogenic variants”. Targetable GAs are actionable GAs, excluding KIT and PDGFRA, which can be treated with drugs approved for GIST or other types of cancer or have shown efficacy in clinical trials for either GIST or different types of cancer.

Results: Up to April 2023,144 patients with advanced GIST were enrolled into the C-CAT database. As platforms for the CGP tests, NOP was performed in eight (6%), F1 in 136 (94%). Fifty-four patients (38%) underwent CGP tests using specimens obtained before initiating treatment with imatinib and 90 (63%) using specimens obtained after imatinib. A total of 119 patients (83%) were in the KIT/PDGFRA group, while 25 patients (17%) were in the wild-type group. Actionable GAs were detected in 95 patients (80%) in the KIT/PDGFRA group and 22 patients (88%) in the wild-type group. In the KIT/PDGFRA group, common actionable GAs were CDKN2A (45%), CDKN2B (34%), RB1 (13%), STK11 (10%), TP53 (7%), with PDGFRA D842V mutation being found in four patients (3%). In the wild-type group, SDHB (36%), TP53 (20%), NF1 (12%), STK11 (12%), SDHA (8%) were common actionable GAs with ETV6-NTRK3 fusion being detected in one patient (2%). Neither group had patients with the BRAF V600E mutated or MSI-high tumors. TMB-high (> 10 Muts/Mb) were observed in one patient (1%) in the KIT/PDGFRA group and in two patients (8%) in the wild-type group. In the KIT/PDGFRA group, 29 patients (24%) had targetable GAs such as small-scale variants in BRCA2, TSC1, KRAS, PALB2 and ERBB2 amplification. In the wild-type group, 12 patients (48%) had targetable GAs, including small-scale variants in NRAS, PIK3CA, PTEN and NF1. Four patients were proposed for treatment with approved drugs: three patients with TMB-high status for pembrolizumab and one patient with ETV6-NTRK3 fusion for entrectinib or larotrectinib.

Conclusion: The present study highlights that approximately 25% and 50% of patients in each KIT/PDGFRA and wild-type group exhibit targetable GAs, thereby illustrating the clinical utility of CGP tests in identifying therapeutic targets in advanced GISTs.

(P 36) CLINICO-GENOMIC ANALYSIS OF KIT/PDGFRA/SDH WILD-TYPE GASTROINTESTINAL STROMAL TUMORS IDENTIFIES POTENTIAL DRIVER MUTATIONS

Ryan A. Denu, MD, PhD[1]; Cissimol Joseph, APRN[1]; Elizabeth Urquiola, PA-C[1]; Precious Byrd, APRN[1]; Ravin Ratan, MD[2]; Maria A. Zarzour, MD[1]; Anthony P. Conley, MD[1]; Dejka M. Araujo, MD[2]; Vinod Ravi, MD, MS, MBA[2]; Shreyaskumar Patel, MD[2]; Wei-Lien Wang, M.D[2]; Alexander J. Lazar, MD, PhD[2]; Neeta Somaiah, MD[2], (1) MD Anderson Cancer Center, Houston, TX, (2) The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: Gastrointestinal stromal tumor (GIST) is the most common mesenchymal neoplasm of the gastrointestinal tract. The vast majority of GISTs (85-90%) are driven by activating mutations in KIT or PDGFRA, and the next most common alterations are in components of the succinate dehydrogenase (SDH) complex (SDHA, SDHB, SDHC, and SDHD genes). A small fraction of GISTs lack alterations in KIT, PDGFRA, and the SDH family. We characterized the clinical and genomic characteristics of these so-called “triple negative” GISTs.

Methods: We searched for patients seen at MD Anderson with diagnosis of GIST and available clinical genomic profiles to identify “triple negative” patients.

Results: Of the 16 patients identified, 9 (56.3%) had gastric and 7 (43.7%) had small intestinal primary sites. Median follow-up from time of diagnosis was 68 months (mean 124.2 months). Fourteen patients (87.5%) eventually developed recurrent or metastatic disease, and 5 of these presented with de novo metastatic disease. For the 9 patients with localized disease initially that later developed recurrent/metastatic disease, the median time to recurrence/metastasis was 29.2 months (range 2.2-509.9 months). For the 11 patients with evaluable response to imatinib (e.g. neoadjuvant treatment or for recurrent/metastatic disease), median PFS with imatinib was 5.2 months (range 0.5-129.4 months). All 16 cases have had clinical genomic sequencing to reveal a potential driver. Four patients were found to have alterations in the RAS/RAF/MAPK pathway: 2 with a BRAF V600E mutation, and 2 with NF1 mutations (1 deletion and 1 splice site mutation). In addition, 2 had PIK3CA mutations (both I391M), 1 had an NTRK3 fusion (ETV6-NTRK3), 1 had a PTEN deletion, 1 had an FGFR1 gain-of-function mutation (K654E), 1 had a CHEK2 loss-of-function mutation (T367fs*), and 1 had an Aurora kinase A fusion (AURKA-CSTF1). The 2 patients with BRAF V600E mutation have both been treated with the combination of BRAF and MEK inhibitors. One patient has been on this therapy for 34.2 months, has had a partial response, and treatment is ongoing; the other patient stopped therapy after 2 months due to toxicity. The patient with ETV6-NTRK3 fusion was treated with larotrectinib for 28.7 months prior to progression, likely due to acquired NTRK3 gatekeeper mutation (F617L) rendering resistance to larotrectinib, then was on a second generation NTRK inhibitor, selitrectinib, for 16.5 months prior to progression.

Conclusion: Triple negative GISTs comprise a diverse cohort with different driver mutations. Compared to KIT/PDGFRA mutant GIST, limited benefit was observed with imatinib in triple negative GIST. In depth molecular profiling can be helpful in identifying driver mutations and guiding therapy.

ID	Age at Diagnosis	Primary Site	Stage at Diagnosis (AJCC 8th)	Site of recurrence/metastasis	Imatinib received in which setting?	PFS on imatinib (months)	Time to relapse from first therapy (months)	Driver	Sequencing Platform (MDA = MD Anderson in-house test)
1	41	Gastric	IIIB	Local	Adjuvant	NA	4.1	PTEN deletion	Neotype, Boston Gene
2	68	Duodenum	IV	Pelvis	Adjuvant	NA	2.4	Unknown	MDA CMS50
3	38	Duodenum	IA	NA	Neoadjuvant	6.7	NA	FGFR1 K654E	MDA STGA 2018
4	21	Gastric	IB	Local, liver	Neoadjuvant and adjuvant	4.1	7.1	Unknown	Foundation One, MDA STGA v2 fusion, MDA KIT and PDGFRA sequencing
5	17	Gastric	IV	Liver	Recurrent/metastatic	129.4	141.8	Unknown	MDA STGA 2018 and fusion panel
6	12	Gastric	Unknown	Right psoas, retroperitoneum	Recurrent/metastatic	28.8	226.2	BRAF V600E	MDA KIT and PDGFRA sequencing, MDA STGA 2018 and fusion panel
7	57	Small intestine	IB	Liver	Adjuvant	NA	57.4	PIK3CA I391M	MDA CMS50
8	44	Gastric	IV	Local, liver, spleen	Recurrent/metastatic	5.7	6.6	ETV6-NTRK3 fusion	Foundation One, MDA liquid biopsy panel v1
9	37	Gastric	IV	Omentum	Neoadjuvant	28	NA	Unknown	Unknown
10	72	Small intestine	IV	Pancreas	Neoadjuvant	5.2	4.4	NF1 deletion	MDA MAPP
11	58	Small intestine	IIIB	Bladder, abdomen	Adjuvant	NA	10.8	BRAF V600E	Single gene assays
12	48	Small intestine	IIIA	Local	Adjuvant	NA	88	PIK3CA I391M	MDA CMS50
13	14	Gastric	Unknown	Liver, lung	Recurrent/metastatic	1.7	509.9	CHEK2 frameshift	Foundation One
14	73	Small intestine	IIIB	Omentum, lymph nodes	Recurrent/metastatic	1	1	AURKA-CSTF1 fusion	Foundation One
15	50	Gastric	Unknown	Local, liver, pancreas, periportal region, duodenum, omentum	Neoadjuvant	0.5	2.2	TP53 loss-of-function	MDA STGA 2018
16	58	Gastric	II	Local	Adjuvant	NA	29.2	NF1 splice site mutation	MDA STGA 2018, MDA MAPP

(P 37) **DISSECTING THE ROLE OF EXTRACELLULAR VESICLES IN THE MANAGEMENT OF GIST**

Alessandro De Vita, PhD[1]; Sofia Gabellone, n/a[2]; Silvia Vanni, PhD[2]; Flavia Foca, N/A, n/a[3]; Valentina Fausti, MD[4]; Giacomo Miserocchi, MSc[5]; Chiara Liverani, PhD[5]; Chiara Spadazzi, PhD[2]; Claudia Cocchi, PhD[1]; Chiara Calabrese, MSc[5]; Davide Cavaliere, MD[6]; Carlo Alberto Pacilio, MD[6]; Giorgio Ercolani, MD[6]; Federica Pieri, MD[7]; Lorena Gurrieri, MD[4]; Nada Riva, MD[8]; Laura Mercatali, PhD[9]; Francesco Fabbri, PhD[9], (1) Istituto Scientifico Romagnolo per lo studio dei Tumori "Dino Amadori", Preclinic and Osteoncology Unit, Meldola, Italy, Meldola, Emilia-Romagna, Italy, (2) Istituto Romagnolo per lo studio dei Tumori "Dino Amadori" IRST IRCCS, Preclinic and Osteoncology Unit, Meldola, Italy, Meldola, Emilia-Romagna, Italy, (3) Unit of Biostatistics and Clinical Trials, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Emilia-Romagna, Italy, (4) Clinical and Experimental Oncology, Immunotherapy, Rare Cancers and Biological Resource Center, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", 47014 Meldola, Italy, Meldola, Emilia-Romagna, Italy, (5) Istituto Romagnolo per lo studio dei Tumori "Dino Amadori" IRST IRCCS, Preclinic and Osteoncology Unit, Meldola, Italy, Bologna, Emilia-Romagna, Italy, (6) General and Oncologic Surgery, "Morgagni-Pierantoni" Hospital, Forlì, Emilia-Romagna, Italy, (7) Pathology Unit, "Morgagni-Pierantoni" Hospital, Forlì, Emilia-Romagna, Italy, (8) Clinical and Experimental Oncology, Immunotherapy, Rare Cancers and Biological Resource Center, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Emilia-Romagna, Italy, (9) Preclinic and Osteoncology Unit, Istituto Scientifico Romagnolo per lo studio dei Tumori "Dino Amadori", Meldola, Emilia-Romagna, Italy

Objective: Gastrointestinal stromal tumors (GIST) represent the most common mesenchymal neoplasm of the gastrointestinal tract. Their biological and clinical behavior can vary from indolent to aggressive depending on different factors including the anatomic location, size and molecular profile. The limited availability of circulating biomarkers which could provide information about the aggressiveness of these rare tumors represents an urgent clinical need in order to guide physicians in the clinical management. Exosomes belong to the group of extracellular vesicles (EV) and play a key role in cellular functions and management of intercellular communication, thus representing a powerful tool for diagnostic applications and clinical settings. To this purpose, we analyzed physiochemical properties and genomic profile of exosomes in a case series of GIST and we correlated the results with the observed aggressiveness features of these malignancies.

Methods: Twenty-five patients affected by GIST were enrolled in the study. Blood sampling of each patients was performed intraoperatively and serum was obtained. Exosomes were isolated from blood serum and characterized by nanoparticle tracking analysis (NTA), dynamic light scattering (DLS) in terms of size, polydispersity, conductivity and zeta potentials, as well as surface antigen profiling using a 37 exosomal surface epitopes biomarkers MACSPlex Exosome Kit. To create a patient clustering, all gathered data were compared to the clinical history of the patients.

Results: The study included 17 GIST with a lesion smaller than 5 cm and 8 GIST greater than or equal to 5 cm. The risk of recurrence was classified as low in 18 patients while 7 patients exhibited an intermediate/high risk. The obtained data showed a no significant stratification for NTA and DLS, in contrast to the results of surface antigen profiling of vesicles analysis. The antigen profiling data showed that an increased expression of CD4 and CD209 antigens correlated significantly with an increased risk of recurrence, while an increased CD14 expression correlated with increased lesion size. A trend of significance was observed in the correlation between the up regulation of CD1c, ROR1, MCSP1 and CD86 and the increase of the risk of recurrence. Moreover, up regulation of HLA-DR, ROR1, CD86, CD14 was significantly associated with combined parameter of increased risk of recurrence and larger tumor size, whereas a trend of significance was observed with CD133/1.

Conclusion: These preliminary results suggest the promising use of circulating vesicles as a prognostic tool for the management of patients with GIST. Notably, we include evidence of the importance of surface antigen profiling of exosomes for patient stratification in terms of tumor aggressiveness and recurrence risk. Genomic analyses are currently underway to investigate the correlation between differentially expressed antigens and inflammatory signaling pathways associated with the presence of macrophages. Future investigation on a wider cohort including additional genomic analyses could better define the biological role of circulating exosomes as promising biomarkers for the management of GIST.

(P 38) EFFICACY AND SAFETY OF RIPRETINIB IN THE TREATMENT OF ADVANCED GASTROINTESTINAL STROMAL TUMORS: A MULTICENTER RETROSPECTIVE STUDY

Yuqiang Du, MA[1]; Tian Hongkun, n/a[1]; Ye Shengwei, doctor[2]; Feng Yongdong, doctor[3]; Jiang Qi, master[4]; Liu Weizhen, doctor[5]; Zeng Xiangyu, doctor[5]; Kaixiong Tao, MD[5]; peng zhang, MD[5], (1) Huazhong University of Science and Technology, wuhan, Hubei, China (People's Republic), (2) Department of Gastrointestinal Surgical Oncology, The Hubei Cancer Hospital, Huazhong University of Science and Technology, wuhan, Hubei, China (People's Republic), (3)

Department of Gastrointestinal Surgery Center and Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, wuhan, Hubei, China (People's Republic), (4) Department of Gastrointestinal Surgery, Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, wuhan, Hubei, China (People's Republic), (5) Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, wuhan, Hubei, China (People's Republic)

Objective: Ripretinib is a switch-control tyrosine kinase inhibitor (TKI) that broadly inhibits KIT and PDGFRA mutations including the multiple primary and secondary mutations that drive disease progression and drug resistance in GIST. In the INVICTUS phase 3 study (NCT03353753) and the bridging phase 2 study conducted in China (NCT04282980), ripretinib demonstrated a statistically significant improvement in median progression-free survival and overall survival with an acceptable safety profile in patients with ≥4th-line GIST. Ripretinib was approved for ≥4th-line therapy of advanced GIST in China in March 2021. However, due to its expensive price, there are limited clinical applications and research reports in China. This retrospective study aims to investigate the efficacy and safety of ripretinib among Chinese patients.

Methods: Clinical data of advanced GIST patients treated with Ripretinib from three hospitals in Hubei Province from April 2021 to January 2023 were retrospectively collected, including lines of treatment lines, efficacy, drug discontinuation and safety profile. The efficacy was determined by overall survival (OS) and cancer specific survival (CSS). Safety was assessed by the incidence and severity of adverse events (AEs).

Results: A total of 29 patients were enrolled, of which 19 were male (65.5%) and 10 were female (34.5%); the median age at the time of ripretinib administration was 60 (28~85) years. Ripretinib was used as a fourth-line treatment in 15 cases (51.7%), third-line treatment in 11 cases (37.9%), and second-line treatment in 3 cases (10.4%). In the first evaluation after ripretinib treatment in 29 patients, partial response (PR) was observed in 5 cases, stable disease (SD) in 16 cases, and progressive disease (PD) in 8 cases. The objective response rate (ORR) was 17.2%, and the disease control rate (DCR) was 72.4%. Adverse events were observed in 25 patients (86.2%), including grade 1-2 adverse events in 23 cases (79.3%) and grade 3 adverse events in 2 cases (6.9%). By the last follow-up, 14 out of 29 patients had died. The 1-year OS rate was 56.4%, and the 1-year CSS rate was 62.1% for the entire group of patients. 11 patients discontinued ripretinib treatment, with 1 patient due to gastrointestinal bleeding and 10 patients due to financial reasons, among which 4 cases experienced disease progression, and the doctor recommended increasing the dose of ripretinib, but the patients chose to discontinue ripretinib due to economical burden. Compared to patients who discontinued ripretinib, those who continued treatment had a significantly longer 1-year OS rate (69.6% vs. 36.4%, $P<0.01$) and 1-year CSS rate (80.4% vs. 36.4%, $P<0.01$)

Conclusion: Ripretinib can effectively control the disease progression of advanced GIST in patients who have failed first-line or multiple-line treatments, with mild adverse events and good tolerability. Compared to patients who discontinued ripretinib, those who continued treatment had more significant survival benefits. Financial reasons were the main factors leading to treatment discontinuation. The inclusion of ripretinib in national reimbursement drug list may bring substantial benefits to patients with advanced GIST.

TABLE 1. Patient Demographics and Baseline Characteristics

Characteristics	n	（%）
Sex		
Male	19	65.5
Female	10	34.5
Age		
≤60 years	16	55.2
＞60 years	13	44.8
Site of primary tumor		
Gastric	12	41.4
Small intestine (including duodenum, jejunum, and ileum)	12	41.4
Colon/rectum/appendix	5	17.2
ECOG score		
0~1	24	82.8
≥2	5	17.2
Lines of treatment		
Fourth-line	15	51.7
Third-line	11	37.9
Second-line	3	10.4
Metastasis		
Hepatic metastasis	19	65.5
Hepatic metastasis with peritoneal metastasis	10	34.5
Mutation biomarkers		
KIT 11	8	27.6
KIT 9	4	13.8
KIT 11+17	9	31.1
KIT 11+13	7	24.1
Wild type	1	3.4

Table 2: Treatment-related treatment-emergent adverse events

Adverse Event	Grade 1–2	Grade 3
Alopecia	17（58.6）	0
Fatigue	11（37.9）	0
Myalgia	7（24.1）	0
Hypertension	4（13.8）	2（6.9）
Palmar-plantar erythrodysesthesia	6（20.7）	0
Constipation	4（13.8）	0
Diarrhoea	2（6.9）	0
Abdominal pain	2（6.9）	0
Nausea	2（6.9）	0
Edema	1（3.4）	0
Gingival bleeding	1（3.4）	0

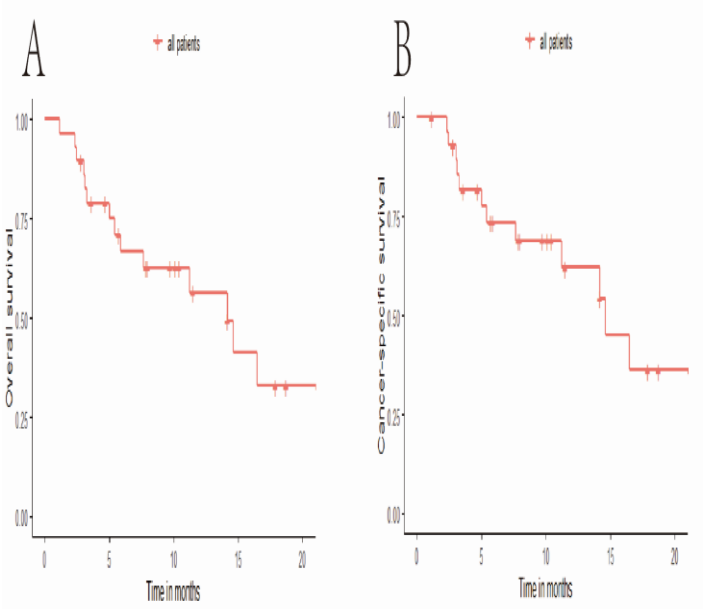


Figure1: Survival curve of all patients. (A) overall survial (B) cancer-specific survival

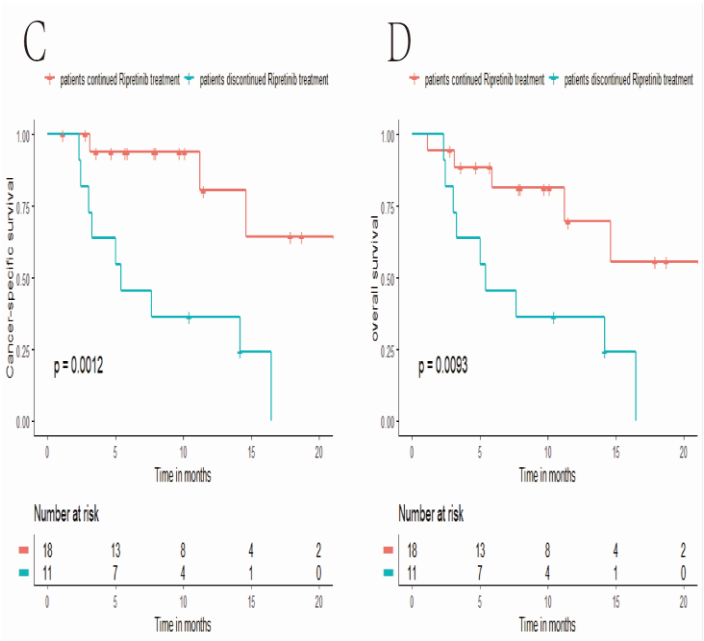


Figure2: Survival curve of patients continued and discontinued Ripretinib treatment. (A) overall survial (B) cancer-specific survival

(P 39) MANAGEMENT PRACTICES OF GASTROINTESTINAL STROMAL TUMORS: A QUESTIONNAIRE SURVEY OF GENERAL SURGEONS IN CHINA

peng zhang, MD[1]; ming wang, MD[2]; tao wang, MD[3]; fan feng, MD[4]; maojun di, MM[5]; yan zhao, MD[6]; Han Liang, MD, PhD[7]; weidong jin, MD[8]; jun zhang, MD[9]; yang fu, MD[10]; jiansi chen, MD[11]; Jian Li, MD, PhD[12]; Haoran Qian, MD[13]; Xinhua Zhang, MD, PhD[14]; leping li, MD[15]; kuntang shen, MD[16]; yong li, MD[17]; yingjiang ye, MD[18]; hui cao, MD[19]; Kaixiong Tao, MD[1], (1) Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, wuhan, Hubei, China (People's Republic), (2) Department of Gastrointestinal Surgery, Renji Hospital, Shanghai Jiaotong University School of Medicine, shanghai, Shanghai, China (People's Republic), (3) Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, hubei, Hubei, China (People's Republic), (4) Division of Digestive Surgery, Xijing Hospital of Digestive Diseases, The Air Force Medical University, xi'an, Shanxi, China (People's Republic), (5) Department of Gastrointestinal Surgery, Taihe Hospital，Hubei University of Medicine, shiyan, Hubei, China (People's Republic), (6) Department of Stomach Surgery, Affiliated Cancer Hospital of Dalian Technological University; Liaoning Cancer Hospital and Institute, shenyang, Liaoning, China (People's Republic), (7) Department of Gastric Surgery, Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer; Tianjin Key Laboratory of Digestive Cancer, Tianjin; Tianjin's Clinical Research Center for Cancer, Tianjin, 300060, P. R. China, Tianjin, Tianjin, China (People's Republic), (8) Department of General Surgery, General Hospital of Central Theater Command, wuhan, Hubei, China (People's Republic), (9) Department of Pharmacy, The First Affiliated Hospital of Chongqing Medical University, Chongqing, Chongqing, China (People's Republic), (10) Department of Gastrointestinal Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China (People's Republic), (11) Department of Medical Image Center, Affiliated Tumor Hospital of Guangxi Medical University, nanning, Guangxi, China (People's Republic), (12) Department of Gastrointestinal Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital & Institute Beijing 100142, P. R. China, Beijing, Beijing, China (People's Republic), (13) Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, hangzhou, Zhejiang, China (People's Republic), (14) Department of Gastrointestinal Surgery, The First Affiliated Hospital, Sun Yat-sen University, guangzhou, Guangdong, China (People's Republic), (15) Department of Gastrointestinal Surgery，Shandong Provincial Hospital Affiliated to Shandong First Medical University, jinan, Shandong, China (People's Republic), (16) Department of Gastrointestinal Surgery , Zhongshan Hospital, Fudan University, shanghai, Shanghai, China (People's Republic), (17) Department of General Surgery，The Fourth Hospital of Hebei Medical University, shijiazhuan, Hebei, China (People's Republic), (18) Department of Gastrointestinal Surgery, Peking University People's Hospital, beijing, Beijing, China (People's Republic), (19) Department of Gastrointestinal Surgery, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, shanghai, Shanghai, China (People's Republic)

Objective: Since Imatinib was approved by the U.S. Food and Drug Administration (FDA) for GIST treatment, the survival period of patients with GIST receiving standardized treatment has been significantly prolonged. GIST are expected to gradually enter the category of "chronic diseases." Therefore, it is necessary to implement a whole-process management for patients with GISTs.To obtain information on the whole-process management concept of GIST among general surgeons in China，we conducted a national questionnaire survey.

Methods: In this survey study, a questionnaire (41 questions) was distributed online between

May 1 and June 30, 2021, among the general surgeons to evaluate the status of the whole-process management concept of GIST.

Results: In total, 697 valid questionnaires were completed. Among the survey participants, 64 (9.2%) were residents, 172 (24.7%) were surgeons-in-charge, 235 (33.7%) were associate-chief surgeons, and 226 (32.4%) were chief surgeons. In the past five years, 122 (32.9%), 76 (20.5%), 76 (20.5%), and 97 (26.1%) hospitals had GIST laparoscopic surgery rates of >75%, 50–75%, 25–50%, and ≤ 25%, respectively. In terms of genetic testing, 248 (66.8%) hospitals had KIT/PDGFRA genetic testing capability. Among the surveyed hospitals, 48 (12.9%) had GIST-specialized outpatient clinics and 213 (57.4%) centers regularly conducted multidisciplinary team (MDT) discussions on GIST. Ninety-one (24.5%) hospitals established a GIST database and 93 (25.1%) hospitals regularly held GIST patient education lectures. Three hundred and nine (83.3%) hospitals regularly followed-up with patients with GISTs. Univariate analysis showed that tertiary hospitals and hospitals with an annual number of GIST operations of >50 cases had better GIST whole-process management concepts than other hospitals, while western hospitals had poorer GIST whole-process management concepts ($P < 0.05$).

Conclusion: While Chinese general surgeons have an understanding of the whole-process GIST management concept, there is a gap between guideline adherence and clinical practice. The corresponding population should be strengthened with precision training for promoting GIST whole-process management concepts is key, and influencing factors, such as hospital level, regional economy, and hospital's annual number of GIST operations, should be considered for decision-making.

(P 40) LONG TERM OUTCOMES OF NEOADJUVANT IMATINIB AND SURGICAL RESECTION OF RECTAL GASTROINTESTINAL STROMAL TUMOURS

Michelle J. Wilkinson, FRCS, PhD[1]; Jaye Broomfield, MBBS[1]; Paris Tekkis, FRCS[1]; charlotte Benson, MBChB, FRCP[1]; Robin L. Jones, BSc, MB BS, MRCP, MD[2]; Christina Messiou, MRCP, FRCP. MD[1]; Jonathan Hannay, FRCS, PhD[1]; Dirk Strauss, FRCS[1]; Andrew J. Hayes, MBBS FRCS, PhD[3]; Myles JF Smith, FRCSI, FRCS, PhD[1], (1) The Royal Marsden Hospital, London, England, United Kingdom, (2) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (3) Royal Marsden Hospital, London, England, United Kingdom

Objective: Rectal gastrointestinal stromal tumours (GISTs) are rare neoplasms that present unique challenges in terms of diagnosis and management. This study aimed to evaluate the long-term outcomes after surgical resection for rectal GISTs.

Methods: A retrospective analysis was conducted of patients with histologically confirmed rectal GISTs treated with surgery at a single institution between 2009-2019.

Results: 18 patients were included. The median age was 51 (range 34-68) years and 61% (n= 11) were female. Neoadjuvant imatinib was used in 11 patients with a median reduction in tumour size of 3 (0-5.3) cm and time to maximum response of 13 (5-39) months. 7 patients had upfront surgery. Median tumour size overall was 6.9 cm (2-14cm) and was similar between the neoadjuvant imatinib 6.8 cm (pre-treatment) and upfront surgery cohort 7 cm.

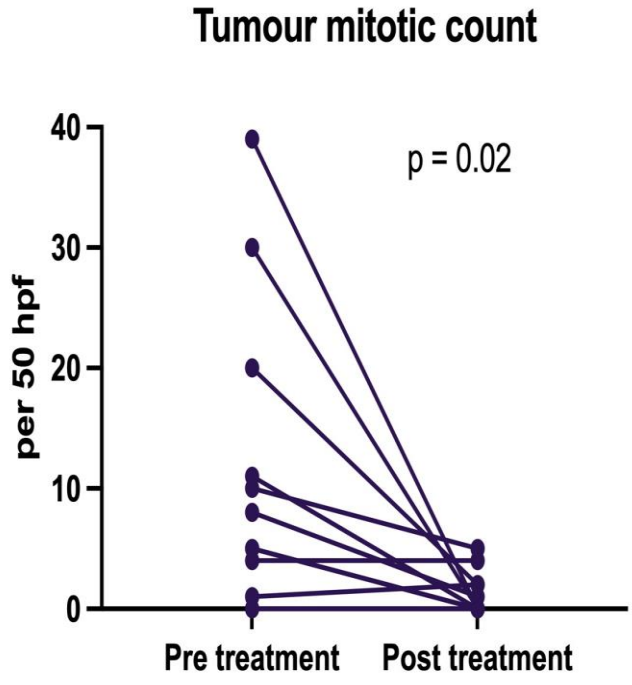
Adjuvant imatinib was used in 11 patients (7 of the neoadjuvant and 5 in the upfront surgery cohort). Median tumour size of patients not given adjuvant imatinib was 2.6 (range 2 - 8) cm. Median tumour mitotic count per 50 high-power fields (50 hpf) was 10 (0-50) overall with 19 (2-50) in the upfront surgery group and 8 (0-40) in the neoadjuvant cohort. After neoadjuvant imatinib there was a statistically significant decrease in tumour mitotic rate p= 0.02 (two-tailed paired t test), median 0 (0-5) 50 hpf.

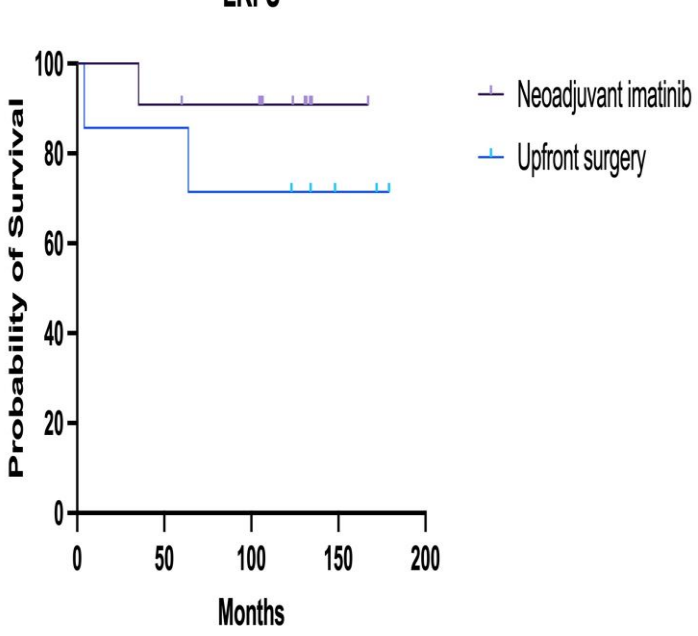
Median follow up was 124 (range 60-179) months, with a median disease free survival (DFS) 117 (4-172) months. The local recurrence rate was 17% (3/18) overall with 9% (1/11) in the neoadjuvant and 29% (2/7) in the upfront surgery cohort. Median time to local relapse was 35 (range 4-64) months. At 5yrs DFS was 83% overall, with 91% and 72% for the neoadjuvant and upfront surgery, respectively. At 7 years the DFS was 71% overall, with 80% and 57 % for the neoadjuvant and upfront surgery, respectively. The overall survival at 7 years was 100% and 83% at 10 years.

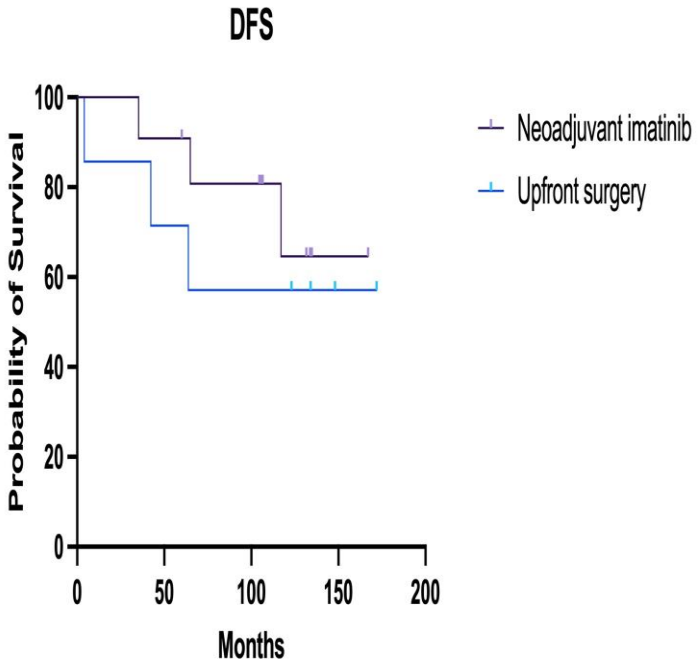
Conclusion: Management of rectal GISTs remains challenging due to the complex location of these tumours and the lack of standardized treatment approaches. Our findings suggest that patients treated with surgical resection can derive durable survival. In our series, the outcome following neoadjuvant imatinib was favourable.

	All	Neoadjuvant imatinib & surgery	Upfront surgery
No pf pts	18	11	7
Age	51 (34 – 68)	60 (34-68)	44 (35-57)
Gender	F: 11 M: 7	F: 4 M: 7	F: 7 M: 0
Pre op maximum diameter (cm)	6.9 (2 – 14)	6.95 (2 – 11.7)	6.31 (2 – 14)
Exon 11	11	7	4
Exon 9	4	2	2
Unknown	3	2	1
Tumour size cm	6.9 (2-14)	Pre imatinib = 6.8 (2-11.7) Post imatinib = 3.6 (1.4-7.8)	7 (2.2-14)
Mitotic count per 50hpf	10 (0-50)	Pre imatinib = 8 (0-40) Post imatinib = 0 (0-5)	19 (2-50)
Type of surgery	11 Local excision 3 AR 4 APR	6 Local excision 2 AR 3 APR	5 Local excision 1 low AR 1 APR
R0/R1 resection	17 (94%)	11 (100%)	6 (86%)
Adjuvant imatinib	11	7	5
Median FU	124	127	134
LR	3/18 (17%)	1/11 (9%)	2/7 (29%)
DM	3/18 (17%)	2/11 (18%)	1/7 (14%)
OS 5 years 7 years 10 years	 100% 100% 83%	 100% 100% 67%	 100% 100% 100%
DFS 5 years 7 years 10 years	 83% 71% 67%	 91% 80% 50%	 72% 57% 50%

Figures given as median and (range) or percentages (%). Anterior resection (AR), Abdominoperineal resection (APR).


Tumour mitotic count
p = 0.02
per 50 hpf
40
30
20
10
0
Pre treatment
Post treatment


LRFS
Probability of Survival
100
80
60
40
20
0
0
50
100
150
200
Months
Neoadjuvant imatinib
Upfront surgery



(P 41) GENOMIC ANALYSIS OF SECONDARY KIT MUTATIONS IN CHINESE PATIENTS WITH IMATINIB-RESISTANT ADVANCED GASTROINTESTINAL STROMAL TUMORS USING NEXT-GENERATION SEQUENCING (NGS)

Haoran Qian, MD[1]; Tianyu Lin, MD[1]; Na Yan, MD[2]; Dan Shen, MD[2]; Xuan Zhang, MD[2]; Yunyun Zhang, MD[3], (1) Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, hangzhou, Zhejiang, China (People's Republic), (2) Key Laboratory of Digital Technology in Medical Diagnostics of Zhejiang Province, Dian Diagnostics Group Co., Ltd., hangzhou, Zhejiang, China (People's Republic), (3) Zailab pharmaceuticals, shanghai, Shanghai, China (People's Republic)

Objective: Gastrointestinal stromal tumors (GISTs) are primarily driven by the abnormal activation of proto-oncogenes KIT/PDGFRA. Although imatinib significantly improved the prognosis of GIST patients, resistance caused by secondary mutations poses a considerable challenge for the treatment of advanced GIST. Secondary mutations often exhibit various types and numbers, with the types and frequencies of such mutations differing based on the primary mutations. As sunitinib and regorafenib are only effective against certain secondary mutations, ripretinib can broadly inhibit a wide range of primary and secondary KIT mutations. Consequently, identifying the secondary mutations of imatinib-resistant GIST patients is crucial for treatment selection in second- and later-line settings. This study aims to analyze the resistance mutation status of Chinese advanced GIST patients who failed first-line imatinib treatment and to investigate the differences in resistance mechanisms resulting from distinct KIT primary mutations.

Methods: Patients with recurrent or metastatic GIST who were admitted to Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University from July 2019 to January 2023 were included. First-line treatment with imatinib was administered to all participants, and surgical or

puncture tissue samples were subsequently collected. NGS was performed on tissue samples with a tumor cell proportion exceeding 20% in the rapidly progressing metastatic lesions. The analysis focused on mutations in nine genes, namely KIT, PDGFRA, KRAS, BRAF, NF1, SDHA, SDHB, SDHC, and SDHD. Additionally, relevant clinicopathological characteristics of the patients were recorded. Approval for this study was granted by the Internal Review Board of Sir Run Run Shaw Hospital.

Results: A total of 69 patients with recurrent or metastatic GIST were administered first-line imatinib treatment. Post-treatment, 65 surgical biopsies and 4 needle biopsies were collected. Among the surgical specimens, 42 (64.6%) were imatinib-resistant while 23(35.4%) were non-resistant. Of the 42 patients exhibiting imatinib resistance, 61.9% were male and 83.3% were aged over 55 years. The primary tumor site was predominantly the small intestine in 66.7% (n=28) and stomach in 7.1% (n=3). Additionally, 52.4% of these patients had a tumor diameter of 5 cm or greater. Primary KIT Exon 9 and KIT Exon 11 mutations were observed in 23.8% (10/42) and 76.2% (32/42), respectively. Among patients with KIT Exon 9 mutations, 20% (2/10) had secondary mutations, all of which were KIT Exon 17 mutations. For patients with KIT Exon 11 mutations, 84.3% (27/32) had secondary mutations, distributed as follows: 33.3% (9/27) in KIT Exon 13, 3.7% (1/27) in Exon 14, 51.9% (14/27) in Exon 17, and 11.1% (3/27) in Exon 18.

Conclusion: The proportion of secondary mutations in KIT Exon 11 primary mutations is significantly higher than that in KIT Exon 9 primary mutations following progression on first-line imatinib treatment. Furthermore, the main resistance mechanism of KIT Exon 11 mutant GISTs continues to rely on complex secondary mutations in KIT signaling. Differences in resistance mechanisms among various primary mutations imply that subsequent treatment should take into account both the patient 's primary and resistance mutations.

(P 42) THE EFFICACY AND SAFETY OF RIPRETINIB IN THE TREATMENT OF CHINESE PATIENTS WITH ADVANCED GASTROINTESTINAL STROMAL TUMORS (GISTS): A SINGLE-INSTITUTION ANALYSIS

Li Zhang, MD, PhD[1]; Xiaona Wang, MD, PhD[2]; Hongjie Zhan, MD, PhD[2]; Song Gao, MD, PhD[3]; Ting Deng, MD, PhD[4]; Baogui Wang, MD, PhD[1]; Ning Liu, MD, PhD[1]; Huikai Li, MD, PhD[5]; Xin Yue, MD, PhD[6]; Rupeng Zhang, MD, PhD[1]; Han Liang, MD, PhD[1], (1) Department of Gastric Surgery, Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer; Tianjin Key Laboratory of Digestive Cancer, Tianjin; Tianjin's Clinical Research Center for Cancer, Tianjin, 300060, P. R. China, Tianjin, Tianjin, China (People's Republic), (2) Department of Gastric Surgery,Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer; Tianjin Key Laboratory of Digestive Cancer, Tianjin; Tianjin's Clinical Research Center for Cancer, Tianjin, 300060, P. R. China, Tianjin, Tianjin, China (People's Republic), (3) Department of Pancreatic Surgery,Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer; Tianjin Key Laboratory of Digestive Cancer, Tianjin; Tianjin's Clinical Research Center for Cancer, Tianjin, 300060, P. R. China, Tianjin, Tianjin, China (People's Republic), (4) Department of gastrointestinal medical oncology,Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer; Tianjin Key Laboratory of Digestive Cancer, Tianjin; Tianjin's Clinical Research Center for Cancer, Tianjin, 300060, P. R. China, Tianjin, Tianjin, China (People's Republic), (5) Department of Hepatobiliary Surgery, Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer;

Tianjin Key Laboratory of Digestive Cancer, Tianjin; Tianjin's Clinical Research Center for Cancer, Tianjin, 300060, P. R. China, Tianjin, Tianjin, China (People's Republic), (6) Department of Colorectal Cancer,Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer; Tianjin Key Laboratory of Digestive Cancer, Tianjin; Tianjin's Clinical Research Center for Cancer, Tianjin, 300060, P. R. China, Tianjin, Tianjin, China (People's Republic)

Objective: Ripretinib, a broad-spectrum KIT and PDGFRA switch-control TKI (Smith et al., 2019), is approved for the fourth-line and above (≥4L) therapy for GISTs. Ripretinib demonstrated comparable overall efficacy and favorable safety versus sunitinib as 2L therapy in INTRIGUE (Bauer S, et al., 2022) and was superior to sunitinib for Chinese patients harboring KIT exon 11 mutations (Li J, et al., 2023), and was recommended as a 2L treatment option by NCCN and CSCO GIST guidelines. Here, we report a clinical practice data evaluating the efficacy and safety of ripretinib in Chinese patients with advanced GISTs.

Methods: Between November 1, 2020 and November 1, 2022, patients with recurrent or metastatic GISTs treated with ripretinib receiving at least one tumor assessment in Tianjin Medical University Cancer Institute & Hospital were prospectively reviewed. The study endpoints were median progression-free survival (mPFS), median overall survival (mOS), objective response rate (ORR), disease control rate (DCR) and safety. Factors associated with the efficacy of ripretinib were aslo analyzed by product limit method (KM).

Results: A total of 23 patients were included in this study. With a median follow-up time of 12.2 months, the mPFS was 11.0 months (95% CI: 8.5, NA) and mOS was 27.0 months (95% CI: 11.0, NA). 3 patients achieved PR and 18 patients achieved SD, and the ORR and DCR were 13% and 91%, respectively. For patients with KIT exon 11 mutations, the mPFS was 12.0 months (95% CI: 8.5, NA) and mOS was 27.0 months (95% CI: 12.9, NA). Compared with clinical trials, the use of ripretinib was more complex in real world. 3 patients discontinued ripretinib due to financial reasons, and 2 patients underwent surgery after 4 and 5 months with ripretinib respectively and discontinued ripretinib after surgery. Although the recommended dose of ripretinib was 150 mg QD, 8/9 patients (one dose reduction) were treated with 100 mg QD for economic reasons. The mOS of patients received 100 mg QD vs 150 mg QD were 11.0 months (95% CI: 6.9, NA) vs 27.0 months (95% CI: 12.9, NA) (P = 0.15, trending in Difference). The starting lines of ripretinib also had an impact on survival data. The mPFS of ripretinib as 2L and 3L therapies vs ≥4L therapy were 14.0 months vs 8.5 months (P < 0.05) and the mOS were not reached vs 11.0 months (P = 0.07, trending in Difference). Other analyses showed that duration of therapy（DOT）of imatinib did not seem to affect the survival data of ripretinib, while DOT of sunitinib seemed to have a trend to affect OS and DOT of regorafenib affected both the PFS and OS of ripretinib. Ripretinib was associated with a favorable safety profile and was also generally well tolerated. After disease progression, 6 patients received ripretinib Intra-patient dose escalation and 4 of the 6 patients achieved SD. 3 patients received combination therapy of ripretinib with other TKIs and 2 of the 3 patients achieved SD. The safety profile for ripretinib dose escalation or combination with other TKIs was acceptable.

Conclusion: Ripretinib has significant clinical benefit and good safety in Chinese patients with advanced GISTs. Earlier use of ripretinib may benefit the survival of the patients as well as for Chinese patients harboring exon 11 mutations. Dose escalation or combination of ripretinib with other TKIs after disease progression may provide additional clinically meaningful benefit with an acceptable safety profile. Further prospective clinical studies with larger sample sizes are needed.

(P 43) UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR-ASSOCIATED PROTEIN AS A POTENTIAL NEXT GENERATION MOLECULAR TARGET FOR TREATMENT OF GASTROINTESTINAL STROMAL TUMOR

Flavia Paternostro, Student[1]; Agnieszka Wozniak, PhD[1]; Chao-Chi Wang, PhD student[1]; Lore De Cock[2]; Luna De Sutter, PhD student[1]; Kimberly Verbeeck, n/a[1]; Daniël Gorgels, n/a[1]; Che-Jui Lee, PhD[1]; Raf Sciot, MD, PhD[3]; Patrick Schöffski, MD, MPH[4], (1) Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Vlaams-Brabant, Belgium, (2) Department of General Medical Oncology, University Hospitals Leuven and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Oost-Vlaanderen, Belgium, (3) Department of Pathology, University Hospitals Leuven, Leuven Cancer Institute, Leuven, Leuven, Vlaams-Brabant, Belgium, (4) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium

Objective: Most gastrointestinal stromal tumors (GIST) are resistant to chemotherapy but highly sensitive to treatment with tyrosine kinase inhibitors. Primary and secondary mutations in KIT and PDGFRA genes, encoding for receptor tyrosine kinases, limit the use of these oral compounds in clinical routine. To overcome or delay the resistance and to develop curative strategies for treatment of GIST, molecular targets beyond traditional receptor kinases may be of interest. Urokinase plasminogen activator receptor-associated protein (uPARAP/Endo180) is a recycling endocytic receptor with a key role in collagen turnover and extracellular matrix homeostasis. In normal tissue, uPARAP is preferentially expressed in cells of mesodermal origin, and the protein is known to be overexpressed in a variety of soft tissue sarcomas (see abstract Wozniak et al. at this meeting). In the current project we aim to explore the molecular epidemiology of uPARAP in GIST, using archival tumor material from GIST patients as well as cell line and patient-derived xenograft material.

Methods: Expression of uPARAP was assessed by immunohistochemistry in GIST specific tissue microarrays (TMA) readily available in our Laboratory. We selected one clinical TMA, and six TMAs from 13 patient-derived and three cell line-derived GIST xenografts from our XenoSarc platform. For these models at least two passages of each tumor were included in the TMAs, with 2–3 cores per passage (523 cores in total). All these models have KIT or PDGFRA driver mutation and five of them harbor secondary mutations. The clinical GIST TMA is constructed from 71 GIST patients (76 samples, 2 cores per each) with different molecular background: primary mutation in KIT exon 11 (44%), PDGFRA exon 18 (17%) and KIT exon 9 (13%), secondary mutation in KIT exon 13 (8%) and KIT exon 17 (8%), and wild type (10%). Xenograft TMAs were manually immunostained with Goat Anti-Human Mrc2 Polyclonal Antibody (R&D systems, cat. No #AF5770). Primary antibody incubation with a 1:100 dilution was performed overnight at 4 °C. Histologic evaluation was performed by estimating the percentage of positive cells (0-100%) and the expression intensity (0-3+) of each core. High uPARAP expression was defined as intensity score of 2 or 3 in ≥ 50% of tumor cells in at least 2 cores. The clinical TMA will be stained with MRC2 Mouse Monoclonal Antibody (clone OTI9G4; OriGene Technologies, cat. No #TA811858).

Results: uPARAP was found to be highly expressed in 9/16 established xenograft models in at least one passage; in three of these models the high expression was preserved across several passages, all harboring a primary KIT exon 11 mutation. Positive models showed only moderate inter-passage variability in uPARAP expression, and positivity was not lost over subsequent passages. Immunostaining and analysis of uPARAP in the clinical GIST TMA are currently ongoing and results will be disclosed at the meeting.

Conclusion: uPARAP is highly expressed in several GIST xenograft models across multiple passages of ex-mouse tumors. Considering its preferential expression in GIST and other mesenchymal tumors and the recycling character of this molecular target, uPARAP may serve as a powerful emerging target for systemic treatment of GIST beyond traditional kinase inhibitors or in combination with the latter. uPARAP positive xenograft models can be used for in vivo evaluation of such anti-uPARAP treatment.

(P 44) RIPRETINIB FOR THE TREATMENT OF CHINESE PATIENTS WITH ADVANCED GASTROINTESTINAL STROMAL TUMORS HARBORING KIT SECONDARY MUTATIONS: A MULTICENTER, RETROSPECTIVE STUDY

Hao Xu, MD[1]; Xiaofeng Sun, MD[2]; Lichun Deng, MD[3]; Wenxian Guan, MD[4]; Xiuping Du, MD[5]; Yefan Zhang, MD[6]; Jia Chen, MD[7]; Lin Liu, MD[8]; Xiufeng Liu, MD[9]; Meifen Qian, MD[10]; Wei Shen, MD[11]; Min Tao, MD[12]; Jun Wu, MD[13]; Guoqiang Zhou, MD[14]; Xinhua Zhang, MD[15]; Ning Liu, MD, PhD[16]; Zekuan Xu, MD[1], (1) Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China (People's Republic), (2) Department of Internal Medicine, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, China (People's Republic), (3) Department of Oncology, The Affiliated Jiangyin Hospital of Nantong University, Jiangyin, Jiangsu, China (People's Republic), (4) Department of General Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China (People's Republic), (5) Department of Medical Oncology, Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China (People's Republic), (6) Department of Hepatobiliary Surgery, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, Beijing, China (People's Republic), (7) Department of Oncology, Jiangsu Cancer Hospital, Nanjing, Jiangsu, China (People's Republic), (8) State Key Laboratory of Pharmaceutical Biotechnology and MOE Key Laboratory of Model Animals for Disease Study, Department of Spine Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China (People's Republic), (9) Department of Medical Oncology of PLA Cancer Center, Jinling Hospital, Nanjing, Jiangsu, China (People's Republic), (10) Department of Medical Oncology, Yixing Hospital of Traditional Chinese Medicine, Wuxi, Jiangsu, China (People's Republic), (11) The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, Jiangsu, China (People's Republic), (12) Department of Oncology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China (People's Republic), (13) Department of Medical Oncology, The First People's Hospital of Changzhou, Changzhou, Jiangsu, China (People's Republic), (14) Department of Gastrointestinal Surgery, Changshu No. 2 Hospital, Suzhou, Jiangsu, China (People's Republic), (15) Department of Gastrointestinal Surgery, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China (People's Republic), (16) Department of Gastric Surgery, Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer; Tianjin Key Laboratory of Digestive Cancer, Tianjin; Tianjin's Clinical Research Center for Cancer, Tianjin, 300060, P. R. China, Tianjin, Tianjin, China (People's Republic)

Objective: Secondary KIT mutations in patients with gastrointestinal stromal tumors (GISTs) are the primary cause of resistance to tyrosine kinase inhibitors (TKIs), and mutational heterogeneity increases with successive lines of therapy. Approximately 70% and 77% of patients develop secondary resistance to first-line imatinib or at least three lines of treatment.

Previous studies indicated that the presence of secondary mutations is associated with reduced median progression-free survival (PFS) in patients receiving sunitinib as second-line (7 vs 19 months) and regorafenib as third-line treatment (approximately 120 vs 170 days). In view of ripretinib is ability to inhibit many common primary and secondary KIT mutations, this study aims to assess the efficacy and safety of ripretinib in Chinese patients with advanced GIST harboring secondary mutations.

Methods: Patients with advanced GIST harboring primary and secondary KIT mutations who received ripretinib 150mg once daily following progression on at least imatinib therapy between June 2, 2020, and Feb 14, 2023, in multicenters were retrospectively analyzed. The PFS, overall survival (OS), objective response rate (ORR), disease control rate (DCR) and safety were assessed and analyzed. The study was approved by the Ethics Committee of the First Affiliated Hospital with Nanjing Medical University.

Results: A total of 31 GIST patients with primary and secondary KIT mutations were evaluated. The median age was 60 years (range, 41–80 years) and the median number of prior lines of therapy was 3 (range, 1-3). The primary tumor predominantly originated in the small intestine (77.42%) and 38.71% of patients with ECOG performance status of ≥2. At the data cut off on June 7, 2023, the median follow-up duration was 16.6 months (95% CI 12.7-20.5). The median PFS was 11.0 months (95% CI, 2.7-19.3), and the ORR and DCR were 22.58%(n=7) and 93.55%(n=29), respectively. The median OS was 19.7 months (95% CI 11.41-27.99). Secondary mutations were identified in 30 patients with KIT exon 11 mutations (13 in exon 13, 15 in exon 17 and 2 in both exon 13 and 17) and in one patient with KIT exon 9 mutation (both exon 13 and 17). The mPFS was 11.0 months (95% CI 3.04-18.96) for the patients with primary KIT exon 11 and secondary mutations. The patient with a primary KIT exon 9 and secondary mutations exhibited a PFS of 21.2 months. Grade 1-2 treatment-emergent adverse events (TEAEs) occurred in 80.65% of patients, and one patient (3.23%) experienced grade 3 palmar-plantar erythrodysesthesia syndrome.

Conclusion: The prevalence of secondary mutations among patients with primary exon 11 mutations seemes higher than those with exon 9 mutations. Ripretinib demonstrated promising mPFS and mOS benefit for patients with both primary and secondary KIT mutations, which was numerically superior to previously reported mPFS for sunitinib and regorafenib in patients with any secondary mutations.

Table 1: Baseline patient characteristics

Patient characteristics	Ripretinib (n = 31)
Age, median (range), years	60（41,80）
<65, n (%)	19（61.3）
≥65, n (%)	12（38.7）
Gender, n (%)	
Male	21 (67.7)
Female	10(32.3)
Site of primary tumor, n (%)	
Stomach	2(6.45)
Small intestine	24(77.42)
Rectum	1(3.23)
Other	4(12.90)
Number of prior treatment, n (%)	
≤ 2	14（45.16）
≥ 3	17（54.84）
Median (min, max)	3（1,3）
Prior treatment regimen, n (%)	
Imatinib	31（100）
Sunitinib	25（80.65）
Regorafenib	17（54.84）
Avapritinib	1（3.23）
ECOG performance status, n (%)	
0-1	19 (61.29)
≥2	12 (38.71)
Site of metastasis, n (%)	
Liver	20（64.52）
Abdominal and pelvic cavity	15（48.39）
Peritoneum	2（6.45）
Other	6（19.35）
Mutation type, n (%)	
KIT exon 11+ exon 13	13（38.46）
KIT exon 11+ exon 17	15（61.54）
KIT exon 11+ exon 13+ exon 17	2（3.85）
KIT exon 9+ exon 13+ exon 17	1（3.85）

1

(P 45) THE LONG-TERM EFFECT OF IMATINIB WITH HEPATIC RESECTION OR OTHER LOCAL TREATMENT FOR GIST LIVER METASTASES

Haoxiang Wen, MM[1]; Yihao Huang[1]; Shaoqing Huang, MM[1]; Han Xiao, MD[1]; Wenxuan Xie, MD[2]; Li Tan, MD[1]; Yuqi Ding, MD[2]; Xiaofei Liu, BM[1]; Yang Yu, MD[2]; Zimin Song, MD[2]; Zebin Chen, MD[2]; Xinhua Zhang, MD, PhD[3], (1) The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China (People's Republic), (2) The First Affiliated Hospital of Sun Yat-sen University, guangzhou, Guangdong, China (People's Republic), (3) Department of Gastrointestinal Surgery, The First Affiliated Hospital, Sun Yat-sen University, guangzhou, Guangdong, China (People's Republic)

Objective: This retrospective study evaluates the long-term survival benefits of combining imatinib mesylate (IM) treatment with hepatic resection (HR) or other local therapies, radiofrequency ablation (RFA), and transarterial chemoembolization (TACE), in comparison to IM treatment alone in patients with liver metastasis from gastrointestinal stromal tumors (GISTs).

Methods: This study included 238 patients diagnosed with liver metastasis of GIST between January 2002 and April 2022 in the First Affiliated Hospital of Sun Yat-Sen University. The oncology outcomes of concern included overall survival (OS) and progress-free survival (PFS) and liver-specific progression-free survival (liver-specific PFS).

Results: Of the 238 patients(Table 1), 126 were treated with IM alone(IM group), 81 with IM combined with HR (IM+HR group), and 31 with IM combined with RFA/TACE (IM+RFA/TACE group). The median follow-up time was 100.3 months. The median OS in the IM group was 132.6 months(Figure 1), while it was not reached in both the IM+HR group and the IM+RFA/TACE group. The 10-year OS rate of the IM+HR group was significantly higher than that of the IM and IM+RFA/TACE groups (91.8% vs 61.1% vs 55.2%, respectively, p=0.015). The liver-specific PFS (p=0.642) and overall PFS (p=0.369) of the three groups showed a

beneficial trend in the combined treatment group. Multivariate analyses revealed that age ≤60 years (HR 0.280, p=0.000) and IM+HR (HR 0.361, p=0.047) were independently associated with better OS (Table 2). In the comparison of IM+HR and IM+RFA/TACE groups, surgical intervention achieving no evidence of disease (NED) was independently associated with better OS (HR 0.099, p=0.034), liver-specific PFS (HR 0.428, p=0.033), and better PFS (HR 0.402, p=0.004)(Table 3,4,5).

Conclusion: In patients with GIST liver metastases, IM combined with HR significantly prolonged OS compared with IM alone and IM combined with RFA/TACE, while OS was similar in IM combined with RFA/TACE and IM alone. Achieving NED status in surgical treatment significantly prolongs the patient's OS, liver-specific PFS, and PFS.

Table 1 Patient baseline clinical characteristics

Parameters	IM (n=128)	IM+HR (n=81)	IM+RFA/TACE (n=51)	P
Age(years)				0.297
≤60	82	61	21	
>60	44	20	10	
Sex ratio(F:M)	42:84	26:55	12:19	0.799
Primary sites				0.496
Stomach	43	32	16	
Small intestine	76	45	13	
Colorectum	3	2	0	
Others	4	2	2	
Metastatic phase				0.064
Synchronous	45	40	15	
Metachronous	83	41	16	
Number of metastases				0.000
≤3	35	52	18	
>3	91	29	13	
Largest diameter of metastases (cm)				0.313
≤3	66	37	19	
>3	60	44	12	
Extrahepatic metastasis				0.687
No	80	56	21	
Yes	46	25	10	
Gene mutation				0.806
KIT_exon 9	14	8	5	
KIT_exon 11	51	40	14	
Wild type	6	4	0	
Others	2	1	1	
Unknown	53	28	11	
IM response before surgical treatment				0.736
CR	–	0	0	
PR	–	8	1	
SD	–	23	11	
Extensive progress	–	2	1	
Limited progress	–	15	7	
NA*	–	53	11	
Objective of surgical treatment				0.018
NED	–	49	11	
non-NED	–	32	20	
Combined with other local therapy				–
No	–	63	–	
RFA	–	15	–	
TACE	–	2	–	
RFA+TACE	–	1	–	

*Not applicable to IM response evaluation

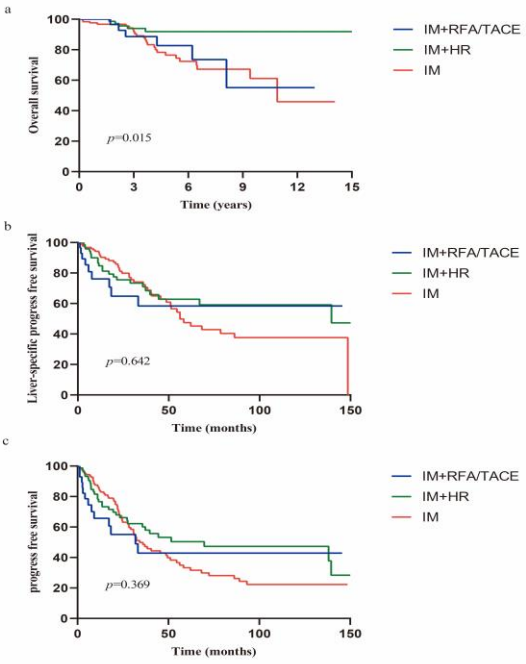


Figure 1 Kaplan–Meier curves (a) overall survival;(b)liver-specific progress free survival;(c) progress free survival.

Table 2 Prognostic factors for overall survival (OS) (IM vs IM+HR vs IM+RFA/TACE)

Parameters	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)	*P*
Gender(Male)	1.587(0.743-3.390)	0.233		
Age(≤60years)	0.275(0.140-0.541)	**0.000**	0.280(0.142-0.555)	**0.000**
Primary Sites(stomach)	0.984(0.489-1.983)	0.965		
Metastatic phase(Metachronous)	0.684(0.352-1.328)	0.262		
Number of Metastases(≤3)	0.431(0.202-0.920)	**0.030**	0.509(0.229-1.131)	0.098
Largest diameter of metastases(≤3cm)	1.263(0.649-2.458)	0.492		
Extrahepatic metastasis（No）	0.661(0.332-1.319)	0.240		
Therapy				
IM alone	Ref	-	Ref	-
IM+HR	0.264(0.100-0.696)	**0.007**	0.361(0.132-0.985)	**0.047**
IM+RFA/TACE	0.887(0.362-2.173)	0.794	1.016(0.404-2.551)	0.974

Table 3 Prognostic factors for overall survival (OS) (IM+HR vs IM+RFA/TACE)

Parameters	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)	*P*
Gender(Male)	1.235(0.327-4.658)	0.756		
Age(≤60years)	0.234(0.070-0.783)	**0.018**	0.395(0.107-1.451)	0.162
Primary Sites(stomach)	2.077(0.631-6.838)	0.239		
Metastatic phase(Metachronous)	0.778(0.237-2.552)	0.678		
Number of Metastases(≤3)	0.511(0.156-1.674)	0.267		
Largest diameter of metastases(≤3cm)	1.305(0.398-4.281)	0.661		
Extrahepatic metastasis（No）	0.358(0.109-1.176)	0.090	0.538(0.148-1.955)	0.346
IM response before surgical treatment (CR+PR+SD+NA vs PD)	0.556(0.163-1.901)	0.349		
Objective of surgical treatment（NED）	0.065(0.008-0.511)	**0.009**	0.099(0.012-0.835)	**0.034**
Therapy（IM+HR）	0.308(0.094-1.012)	0.052	0.625(0.184-2.119)	0.450

Table 4 Prognostic factors for liver specific PFS (IM+HR vs IM+RFA/TACE)

Parameters	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
Gender(Male)	1.163(0.519-2.604)	0.714		
Age(≤60years)	0.459(0.224-0.940)	**0.033**	0.506(0.245-1.048)	0.067
Primary Sites(stomach)	1.539(0.768-3.087)	0.224		
Metastatic phase(Metachronous)	0.738(0.366-1.488)	0.396		
Number of Metastases(≤3)	0.314(0.154-0.640)	**0.001**	0.336(0.162-0.696)	**0.003**
Largest diameter of metastases(≤3cm)	1.202(0.592-2.443)	0.611		
Extrahepatic metastasis（No）	0.584(0.284-1.201)	0.144		
IM response before surgical treatment (CR+PR+SD+NA vs PD)	0.436(0.193-0.985)	**0.046**	0.591(0.255-1.373)	0.221
Objective of surgical treatment（NED）	0.337(0.162-0.701)	**0.004**	0.428(0.196-0.934)	**0.033**
Therapy（IM+HR）	0.733(0.337-1.594)	0.434		

Table 5 Prognostic factors for PFS (IM+HR vs IM+RFA/TACE)

Parameters	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
Gender(Male)	0.971(0.509-1.854)	0.929		
Age(≤60years)	0.487(0.266-0.893)	**0.020**	0.549(0.289-1.043)	0.067
Primary Sites(stomach)	1.344(0.748-2.415)	0.322		
Metastatic phase(Metachronous)	0.836(0.464-1.506)	0.551		
Number of Metastases(≤3)	0.294(0.160-0.541)	**0.000**	0.318(0.168-0.599)	**0.000**
Largest diameter of metastases(≤3cm)	1.023(0.569-1.838)	0.940		
Extrahepatic metastasis（No）	0.384(0.212-0.697)	**0.002**	0.479(0.260-0.883)	**0.018**
IM response before surgical treatment (CR+PR+SD+NA vs PD)	0.555(0.272-1.130)	0.104		
Objective of surgical treatment（NED）	0.353(0.193-0.646)	**0.001**	0.402(0.215-0.751)	**0.004**
Therapy（IM+HR）	0.712(0.373-1.358)	0.302		

(P 46) CIRCULATING TUMOR DNA (CTDNA) ANALYSES AND RESISTANCE MECHANISMS EXPLORATION OF KIT-MUTANT GASTROINTESTINAL STROMAL TUMORS (GISTS) TREATED WITH AVAPRITINIB OR RIPRETINB

Jian Li, MD, PhD[1]; Zhi Dong, MD, PhD[2]; Changsong Qi, MD, PhD[2]; Cheng Zhang, MD, PhD[2]; Lin Shen, MD[3], (1) Department of Gastrointestinal Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital & Institute Beijing 100142, P. R. China, Beijing, Beijing, China (People's Republic), (2) Department of Gastrointestinal Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Peking University Cancer Hospital & Institute Beijing 100142, P. R. China, Beijing, Beijing, China (People's Republic), (3) Department of Gastrointestinal Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Peking University Cancer Hospital & Institute, Beijing, Beijing, China (People's Republic)

Objective: Resistance mechanisms of avapritinib and ripretinib, two novel TKIs for GISTs, have not been fully elucidated. CtDNA NGS-based sequencing is valuable for describing the complex landscape of resistant GISTs, overcoming the problem of heterogeneity. Here, we characterized the genomic heterogeneity of plasma samples from patients with advanced GISTs at baseline and progression of avapritinib or ripretinib to understand the mechanisms of resistance.

Methods: Liquid biopsies (plasma ctDNA) of patients with advanced GISTs in Peking University Cancer Hospital & Institute, were collected predose on Cycle 1 Day 1 and after failure of avapritinib or ripretinib and were profiled using an NGS liquid biopsy assay.

Results: CtDNA analysis at baseline and progression was performed in 25 patients treated with avapritinib. CtDNA was detected in 80% samples, with KIT mutations detected in 60%. A total of 37 KIT mutations were detected in progression samples of 17 patients (mean, 2.18). KIT

exon 17 missense mutations (9/37, 35.1%), KIT exon 9 duplication mutations (6/37, 16.2%) and KIT exon 11 deletion mutations (5/37, 13.5%) were commonly detected. KIT exon 9 duplication mutation rate was significantly increased in progression samples compared with baseline. Meanwhile, novel mutation types that were not present at baseline, such as KIT exon13/14/16 missense mutations, exon13 insertion mutations and exon 8 deletion mutations, were detected in progression samples. In addition to KIT mutations, other common signaling pathway alterations at progression of avapritinib were detected, mainly located in PI3K-Akt pathway and cell cycle pathway, followed by RTK-RAS pathway. Plasma ctDNA was analyzed in 25 patients at baseline and 14 patients after failure of ripretinib. CtDNA was detected in 66.7% samples, with KIT mutations detected in 61.5%. A total of 16 KIT mutations were detected in progression samples of 9 patients (mean, 1.78). KIT exon 11 deletion mutations (3/16), deletion insertion mutations (3/16) and missense mutations (3/16) were commonly detected. Exon 11 missense mutations, followed by KIT exon 14 missense mutations and KIT exon11 deletion insertion mutations were significantly increased in progression samples compared with baseline. The most common signaling pathways activation at progression of ripretinib included TP53-related pathway, RTK-RAS pathway, Cell-cycle pathway, MYC pathway and NOTCH pathway.

Conclusion: Different KIT mutation types and signaling pathways atlerations were detected in progression samples of KIT-mutant GISTs after avapritinib or ripretinib failure compared with baseline, which may be associated with mechanism of resistance.

(P 47) PATIENT SURVIVAL IN METASTATIC GIST WITH IMATINIB-SENSITIVE MUTATIONS: REAL WORLD DATA FROM GERMAN REFERRAL CENTER (2008-2021)

Valerie Haller[1]; Carina Reiff, n/a[1]; Rainer Hamacher, MD[2]; Julia Ketzer[3]; Yasmin Zaun, MD[1]; Karina Kostbade, MD[2]; Buo-Phuc Nguyen, MD[1]; Moritz Kaths, MD, MSc[1]; Juergen Treckmann, MD[1]; Johanna Falkenhorst, MD[2]; Sebastian Bauer, MD[2], (1) University Hospital Essen, Sarcoma Centre, Essen, Nordrhein-Westfalen, Germany, (2) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (3) Sarcoma Center, Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany

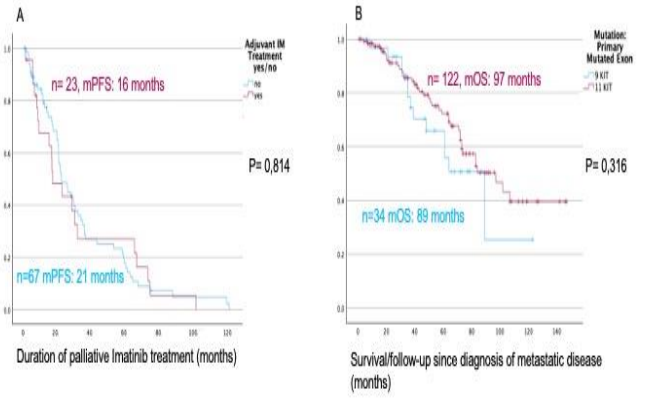
Objective: The outcome of patients with metastatic GIST and imatinib-sensitive primary mutations has dramatically improved. However, published follow-up data from clinical trials commonly excludes elderly and multimorbid patients. In addition, accessibility to drugs may vary between different health care systems. Data to advise patients about outcome at various treatment-lines is scarce. Hence we aimed to analyze the outcome of patients from a large German GIST referral center from the recent 15 years.

Methods: The institutional GIST database (n=773) was queried for patients who were diagnosed with metastatic disease after 2008 att which time at least three lines of treatment were available in Germany. We filtered for patients that harbored imatinib-sensitive mutations as defined by KIT Exon 9 or 11 mutations. In patients with unknown mutational status, response to imatinib treatment for >1 year was defined as Imatinib-sensitive. In total, 163 patients were identified and analyzed for treatment-line specific progression-free survival and overall survival.

Results: The cohort consisted of 70 female and 93 male patients. Median age at diagnosis of metastases was 59 years and median follow-up was 50months. 127 had a primary exon 11 and 36 a primary exon 9 mutation. 58 patients had died of their disease at the time of analysis. The

total patient cohort had a median overall survival (OS) of 84, 44 and 22 months (N= 156, 89 and 82, respectively) after start of palliative imatinib treatment, imatinib failure and sunitinib failure, respectively. Median overall survival was 97 months for patients with primary exon 11 mutation and 89 months in those with a primary exon 9 mutation (p=0,316) (figure B). Notably, the interval between diagnosis of primary tumor and metastatic disease (synchronous vs metachronous diagnosis) did not impact survival. In addition, progression-free survival was identical for imatinib in patients who had received imatinib perioperatively.

Conclusion: In this retrospective cohort we provide real-world data on the outcome of patients in an era of access to multiple kinase inhibitors. The median overall survival by far exceeds historical data for patients with primary exon 11 and exon 9 mutations (8.1 and 7.4 years vs 3.7 years in the meta-GIST analysis for EU patients) which may hint at the survival impact of additional treatment lines since 2008. Notably, adjuvant treatment did not impact time-to-progression and overall survival of patients receiving imatinib for metastatic disease underscoring that apoptotic resistance rather than secondary KIT-mutations are responsible for relapse of patients (figure A).



Survival of patients with Imatinib sensitive metastatic disease and diagnosis after 2008. Figure A: Progression free survival. Figure B: Overall survival.

(P 48) SIGNIFICANCE OF MONITORING PLASMA CONCENTRATION OF RIPRETINIB IN CHINESE PATIENTS WITH GASTROINTESTINAL STROMAL TUMORS

Hao Xu, MD[1]; Xiaofeng Sun, MD[2]; Wenxian Guan, MD[3]; Lichun Deng, MD[4]; Xiufeng Liu, MD[5]; Wei Shen, MD[6]; Jia Chen, MD[7]; Xiaolin Li, MD[8]; Zekuan Xu, MD[9], (1) Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, nanjing, Jiangsu, China (People's Republic), (2) Department of Internal Medicine, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, China (People's Republic), (3) Department of General Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University

Medical School, Nanjing, Jiangsu, China (People's Republic), (4) Department of Oncology, The Affiliated Jiangyin Hospital of Nantong University, Jiangyin, Jiangsu, China (People's Republic), (5) Department of Medical Oncology of PLA Cancer Center, Jinling Hospital, Nanjing, Jiangsu, China (People's Republic), (6) The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, Jiangsu, China (People's Republic), (7) Department of Oncology, Jiangsu Cancer Hospital, Nanjing, Jiangsu, China (People's Republic), (8) Department of Geriatric Gastroenterology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China (People's Republic), (9) Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China (People's Republic)

Objective: Ripretinib, has been approved as a fourth-line and above treatment for advanced gastrointestinal stromal tumors (GIST) with the standard dose of 150 mg daily. Upon disease progression, ripretinib intrapatient dose escalation (IPDE) to 150 mg twice daily was recommended by the NCCN and CSCO guidelines. In phase I study of ripretinib, the peak concentration (Cmax, 502ng/ml) was reached at an average of 4.02 hours (range, 1.95-24.05 hours) after a single dose of 150 mg daily, and on day 15, the Cmax (761ng/ml) was reached at an average of 2.08 hours (range, 1.02-8.13 hours). The steady-state of ripretinib was achieved after 14 days. Currently, no reports exist on blood concentrations of ripretinib during real-world treatment of advanced GIST patients. This study aims to explore the significance of monitoring plasma concentration of ripretinib in the treatment of Chinese GIST patients.

Methods: Advanced GIST patients who received ripretinib treatment from June 2021 to April 2023 were included. In patients with advanced GIST who had reached a steady-state ($>$2 weeks) of ripretinib treatment, peripheral venous blood samples were collected before drug administration and at 4 hours following drug administration. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to detect patients' ripretinib plasma concentrations. Correlations of ripretinib plasma concentration with dosage, clinical efficacy and adverse reactions were analyzed.

Results: A total of 34 patients with 38 specimens were included, with a median age of 59.5 years (range, 33-85 years). The overall mean±SD of the steady-state plasma trough concentrations (Cmin) for ripretinib was 412.62±280.88 ng/mL. The Cmin (mean±SD) were 299 ± 193 ng/mL, 392 ± 253 ng/mL, and 809 ± 431 ng/mL in patients who underwent dose reduction (50 or 100 mg daily), standard dose (150 mg daily), and IPDE (200 mg daily or 150 mg twice daily), respectively. The differences of ripretinib Cmin between standard dose and IPDE group were significant ($P < 0.05$). The median follow-up time was 5.3 months (95% CI, 1.53-9.07 months), and the median progression-free survival (mPFS) is not mature. In patients with assessable efficacy, the objective response rate (ORR) was 6% and 12%, while the disease control rate (DCR) was 71% and 88% for patients with Cmin below or above the mean value, respectively. No significant correlation was observed between ripretinib Cmax and common adverse reactions, including alopecia, fatigue, muscle pain, diarrhea, constipation, and anemia.

Conclusion: The ripretinib Cmin in Chinese GIST patients with dose escalation following disease progression was significantly higher than patients treated with the standard dose. A higher ripretinib Cmin appears to be associated with improved disease control. Furthermore, this study revealed no significant correlation between ripretinib Cmax and common adverse reactions. Further validation is required in the future.

(P 49) EFFICACY OF RIPRETINIB IN A MULTICENTER EXPANDED ACCESS PROGRAM IN PATIENTS WITH ADVANCED GASTROINTESTINAL STROMAL TUMORS HARBOURING NON-KIT EXON 11 MUTATIONS.

Alessandro Mazzocca, MD[1]; Andrea Napolitano, MD[2]; Maria Pantaleo, MD PhD[3]; Giuseppe Badalamenti, MD[4]; César Serrano, MD, PhD[5]; Thibaud Valentin, MD[6]; Heikki Joensuu, Professor[7]; Neeltje Steeghs, Dr.[8]; Peter Reichardt, MD, PhD[9]; Jenny Sherriff, Dr.[10]; Giovanni Grignani, MD[11]; Benjamin Verret, MD[12]; Axel Le Cesne, MD[13]; Mikael Eriksson, MD, PhD[14]; Elena Fumagalli, MD[15]; Robin L. Jones, BSc, MB BS, MRCP, MD[16]; Sebastian Bauer, MD[17]; Bruno Vincenzi, MD, PhD[1], (1) Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Lazio, Italy, (2) The Royal Marsden NHS, London, UK, London, England, United Kingdom, (3) Institute of Hematology and Medical Oncology L & A Seragnoli and Interdepartmental Centre for Cancer Research G Prodi, Bologna, Emilia-Romagna, Italy, (4) Policlinico P. Giaccone, Palermo, Sicilia, Italy, (5) Medical Oncology Department, Hospital Universitario Vall d'Hebron, Vall d'Hebron Institute of Oncology, Barcelona, Spain, Barcelona, Catalonia, Spain, (6) Oncopole Claudius Regaud, Toulouse, Midi-Pyrenees, France, (7) Helsinki University Hospital and University of Helsinki, Helsinki, Finland, Helsinki, Uusimaa, Finland, (8) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (9) Helios Klinikum Berlin-Buch, Medical School Berlin, Berlin, Berlin, Germany, (10) Cancer Centre, Queen Elizabeth Hospital, Birmingham, United Kingdom., Birmingham, England, United Kingdom, (11) Candiolo Cancer Institute, FPO – IRCCS, Turin, Piemonte, Italy, (12) Gustave Roussy, Paris, Ile-de-France, France, (13) Gustave Roussy Institute, Villejuif, Ile-de-France, France, (14) Skane University Hospital, Lund, Skane Lan, Sweden, (15) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (16) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (17) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany

Objective: There remains an unmet need for effective and well tolerated systemic therapy for metastatic gastrointestinal stromal tumors (GISTs) after disease progression on imatinib is challenging. Ripretinib is a novel tyrosine kinase inhibitor with a broad spectrum of activity against KIT and PDGFRA mutations. Patients harbouring KIT exon 11 mutation derive meaningful clinical benefit from ripretinib, but less is known about its activity in other mutational subtypes. We performed a retrospective analysis of advanced GIST patients harbouring primary mutations other than KIT exon 11 and enrolled in the European expanded access program between December 2019 and February 2023.

Methods: Primary efficacy endpoints included were Progression Free Survival (PFS), Overall Survival (OS) and Disease Control Rate (defined as complete remission + partial remission + stable disease) based on Response Evaluation Criteria in Solid Tumors (RECIST).

Results: Overall, 35 patients with advanced GIST and primary mutations other than KIT exon 11, received ripretinib at 14 European centers. Primary mutations were detected in 27 (77.2%) tumor specimens. 19 of them were in KIT (16 exon 9, 2 exon 13, 1 exon 17), 6 in PDGFRA (4 exon 18 D842V and 2 in exon 14) and 2 in SDH genes. Secondary KIT mutations were detected in 4 (11.4%) patients prior to ripretinib treatment (2 exon 13, 1 exon 14, 1 exon 18). Remaining 4 (11.4%) were KIT/PDGFRA/SDH wild type. Median age on commencing ripretinib was 61 (range 21-81) years and most patients were male (21, 60%). The most frequent primary sites were jejunum (17, 48.6%), stomach (9, 25.7) and duodenum (4, 11.4%). In 30 patients (85.7%) ripretinib was used as fourth or later line treatment. Only 9 patients (25.7%) required a

dose reduction while 6 (17.1%) patients were escalated to ripretinib BID after disease progression. The Disease Control Rate (DCR) was 51.4% for the whole population and 66.7% for those patients whose ripretinib dose was escalated. In particular we observed 4 partial responses and 14 stable diseases in patients receiving standar dose of Ripretinib. After a median follow up of 15.4 (95% CI 9.1-NA) months, the median progression free survival was 4.3 (95% CI 3.2-6.2) in those patients receiving Ripretinib standard dose (150 mg QD) and 4.9 (95% CI 1.1-NA) in those who received dose escalation. Median overall survival was 12.2 months (95% CI 5.8-NA). Only 4 (11.4%) patients experienced grade 3 or greater (G ≥ 3) treatment related adverse events (TRAEs). The most common G ≥ 3 TRAEs were arthalgias/myalgias, skin toxicity and asthenia.

Conclusion: In the real world setting, ripretinib has durable benefit in metastatic GISTs harbouring non KIT exon 11 primary mutations. Ripretinib at a dose of 150 mg BD can be safely administered. Ripretinib is generally well tolerated with toxicity profile consistent with previous reports. Due to the small patient numbers, no conclusions can be drawn on the activity of ripretinib for each specific mutational subtype.

(P 50) CHARACTERIZATION OF A FAMILIAL GIST KINDRED INTOLERANT TO IMATINIB

Adam M. Fontebasso, MD, PhD[1]; Katrina M. Ingley, MD[2]; Rebecca Gladdy, MD, PhD[3]; Matthew Zatzman, n/a[1]; Valli Subasri, n/a[1]; Anna Goldenberg, PhD[4]; Elizabeth Demicco, MD, PhD[5]; Abha Gupta, MD MSc[6]; Marta Szybowska, MSc[7]; Seng Thipphavong, MD[8]; David Malkin, MD[9]; Anita Villani, MD[9]; Adam Shlien, PhD, FCCMG[9]; Raymond Kim, MD-PhD[8], (1) University of Toronto, Toronto, ON, Canada, (2) University College Hospital London (UCLH), London, England, United Kingdom, (3) Mount Sinai Hospital, Sinai Health System, and Princess Margaret Cancer Centre Toronto, ON, Canada Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada University of Toronto, Toronto, ON, Toronto, ON, Canada, (4) Sickkids Research Institute, Toronto, ON, Canada, (5) Sinai Health System, Toronto, ON, Canada, (6) Princess Margaret Cancer Center, Toronto, ON, Canada, (7) Fred A. Litwin Family Centre in Genetic Medicine, University Health Network, Toronto, ON, Canada, (8) University Health Network, Toronto, ON, Canada, (9) The Hospital for Sick Children, Toronto, ON, Canada

Objective: OBJECTIVE: Gastrointestinal stromal tumors (GISTs) are rare tumors which can occur throughout the gastrointestinal tract and for which a significant proportion have been found to harbor mutations in the KIT oncogene permitting targeted therapy with the use of imatinib. We sought to characterize the molecular and clinical characteristics of a family with multiple generations affected by multifocal GISTs with imatinib intolerance.

Methods: METHODS: A complete chart review was conducted including clinical, pathologic and radiologic review for the proband, her mother and additional family members where information available and used to construct a pedigree. Tumors were sequenced through the SickKids Cancer Sequencing Program, KiCS (Toronto, Ontario, Canada) on banked frozen tumor tissue from the proband as well as her mother, as well as matched germline blood sample for each patient. Data processing was performed using previously described methods. Somatic mutational burden was assessed for each patient, and whole-genome sequencing data was utilized to assess for copy number changes.

Results: RESULTS: Within our familial kindred affected by multifocal GISTs across

generations, we noted a severe intolerance to imatinib after 8 weeks of therapy. We identified a novel germline variant in KIT not previously c.1965T>G; p.Asn655Lys, not previously seen in population databases but which has been seen as a somatic mutation in jejunal GIST, acral melanoma and acute myeloid leukemia. We also concomitantly identified a germline variant in the MSR1 gene, macrophage receptor scavenger 1, encoding a stopgain variant in exon 6 (c877C>T; p.Arg293*) rs41341748 shared between patients over 3 generations of this family.

Conclusion: CONCLUSIONS: We suspect this novel germline variant of KIT may have resulted in a hereditary predisposition to multifocal GISTs as well as variably penetrant additional phenotypes including altered gut motility and immune dysregulation. The identification of a germline stopgain variant in MSR1 across this familial kindred presents an opportunity for further investigation for additional pathways at play in hereditary cancer development in this family.

Bone Sarcomas

(P 51) OAIE TRIAL IN PROGRESS: A RANDOMIZED PHASE II TRIAL ASSESSING APATINIB IN COMBINATION WITH IFOSFAMIDE AND ETOPOSIDE(IE) VERSUS SINGLE IE CHEMOTHERAPY FOR RELAPSED OR REFRACTORY OSTEOSARCOMA

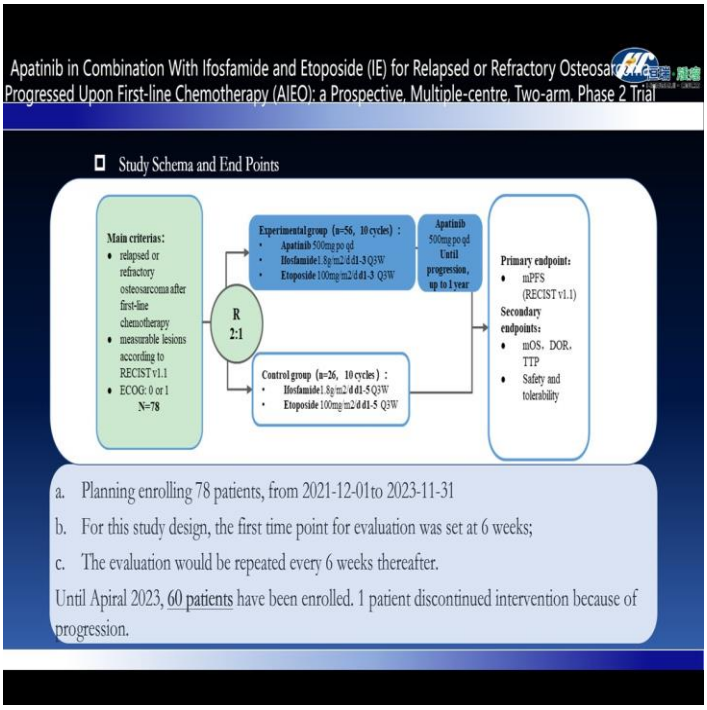
Lu Xie, MD; Jie Xu, MD; Xiaodong Tang, M.D.; Wei Guo, N/A, M.D., Peking University People's Hospital, Beijing, Beijing, China (People's Republic)

Objective: This phase II randomized controlled trial (OAIE) will assess whether the combination of Apa+IE is superior to IE alone in children, adolescents and young adults with relapsed/refractory osteosarcoma.

Methods: OAIE Trial is a national multiple-center, randomized phase II trial in patients with Relapsed or Refractory Osteosarcoma Progressed Upon First-line Chemotherapy [high-dose methotrexate (12 g/m2), doxorubicin (75 mg/m2), cisplatin (100-120 mg/m2) with or without ifosfamide (12 g/m2)]. Treatment arms consist of Apa 500 mg once daily (QD) orally plus Ifosfamide 1.8 g/m2/d d1-3 and etoposide 100 mg/m2/d d1-3 once every 3 weeks (Q3w) versus Ifosfamide 1.8 g/m2/d d1-5 and etoposide 100 mg/m2/d d1-5 once every 3 weeks (Q3w) for at least 10 cycles. The primary objective: to compare median Progression Free Survival (mPFS) between arms (RECIST 1.1, investigator assessed). Secondary endpoints include Objective Response Rate (ORR); Overall Survival(OS); safety; quality of life. Statistical Design: Randomization 2:1; balanced for stratification factors: None. Assuming a mPFS month for patients on the control arm and estimated 6m improvement in mPFS (5m to 11m (HR 0.4545)) with 80% power using a 2-sided 5% level test requires a sample size of 78 including 10% drop out rate. The DSMC reviewed and recommended trial continuation in December 2022. Clinical trial information: NCT05277480.

Results: N/A if you are submitting under Trials in Progress.

Conclusion: N/A if you are submitting under Trials in Progress.



(P 52) ARTEMIS-002 TRIAL IN PROGRESS: A MULTI-CENTER PHASE II TRIAL ASSESSING HS-20093 (B7-H3 ANTIBODY-DRUG CONJUGATE) FOR RELAPSED OR REFRACTORY OSTEOSARCOMA AND OTHER SARCOMAS

Lu Xie, MD; Jie Xu, MD; Xin sun, N/A, MD; Kuisheng Liu, M.D.; Xin Liang, M.D.; Xiaodong Tang, M.D.; Wei Guo, N/A, M.D., Peking University People's Hospital, Beijing, Beijing, China (People's Republic)

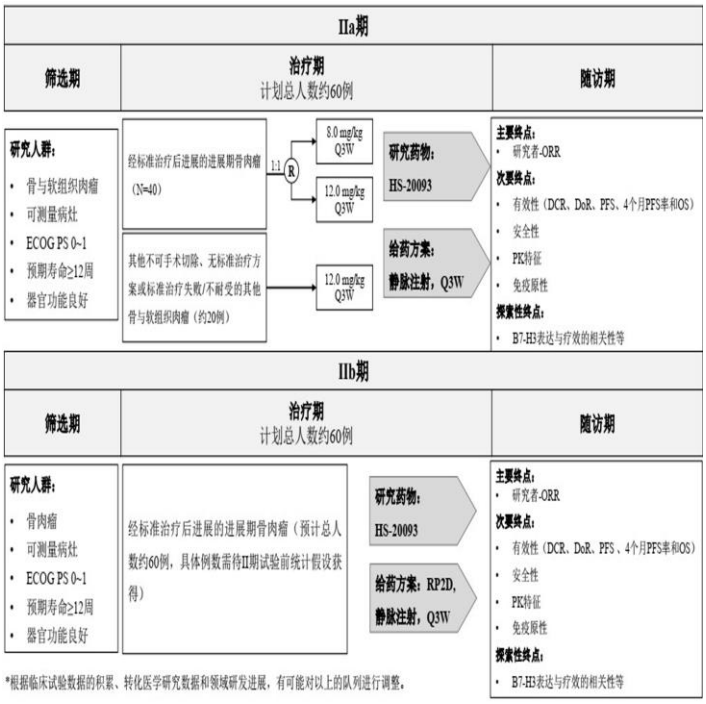
Objective: this phase II trial is intended to investigate the activity of the B7-H3-targeting ADC HS-20093 in these sarcomas, with special attention on osteosarcoma patients.

Methods: ARTEMIS-002 trial is a national multiple-center, two-arm phase II trial in pts with R/R osteosarcoma or other sarcomas progressed upon first-line systemic treatment, which is consisted of two phases. Phase IIa (the proof of concept study) contains two arms, of which one arm contains osteosarcoma only and the other includes all kinds of sarcoma pts. Based on the results of our phase I trial that the maximum tolerated dose (MTD) was 16mg/kg once every 3 weeks (Q3W) intravenous infusion, the osteosarcoma pts in arm one will be randomly assigned 1:1 to receive HS-20093 either at 8 mg/kg or 12 mg/kg Q3W, and the sarcoma pts in arm two will receive 12 mg/kg Q3W. An estimated number of 60 pts will be enrolled in phase IIa trial, with about 20 pts in every dose cohort. We will further identify the better dose based on phase IIa study for osteosarcoma by balancing the efficacy and the safety and use the dose for Phase IIb for about 80 osteosarcoma pts. Key eligibility criteria are age ≥18 years, histologically confirmed osteosarcoma or other sarcomas, ECOG performance status 0-1, measurable disease per RECIST 1.1. The primary endpoint is the objective response rate (ORR) per RECIST 1.1 by independent review committee. Secondary endpoints include disease control rate (DOR), duration of response (DoR), progression free survival (PFS) and overall survival (OS). Safety, pharmacokinetics and immunogenicity will also be assessed as the other secondary endpoints. Exploratory studies are designed to evaluate the correlation between

exposure and responses to HS-20093, and explore the potential efficacy biomarker (B7-H3 protein expression, soluble B7-H3 concentration, PD-L1 protein expression and genetic abnormality) of HS-20093.Clinical trial information: NCT05830123.

Results: N/A Now the trial is enrolling.

Conclusion: N/A Now the trial is enrolling.



(P 53) CURATIVE-INTENT SURGERY FOR A SOLITARY ISOLATED BONE METASTASIS FROM EXTREMITY SARCOMA: WHO UNDERWENT METASTASECTOMY AND WHAT WAS THE PROGNOSTIC OUTCOME?

Liuzhe Zhang, MD[1]; Jean-Camille Mattei, MD[2]; Anthony Griffin, MSc[3]; Kim Tsoi, MD, PhD[4]; Peter Ferguson, MSc., MD[4]; Jay Wunder, M.Sc, MD[4], (1) Mount Sinai Hospital, Department of Surgery, University of Toronto, Toronto, Canada, Toronto, ON, Canada, (2) University Musculoskeletal Oncology Unit, Mount Sinai Hospital, Department of Surgery, University of Toronto, Toronto, Canada, Toronto, ON, Canada, (3) Mount Sinai Hospital, Toronto, ON, Canada, (4) University of Toronto, Toronto, ON, Canada

Objective: Approximately 40% of sarcoma patients will develop lung metastasis, and 10% will develop bone metastasis. The survival benefit of metastasectomy for solitary lung metastasis has been shown, but the role of surgery for solitary bone metastases remains unclear. We investigated the impact of curative-intent surgery on solitary bone metastasis from sarcoma.

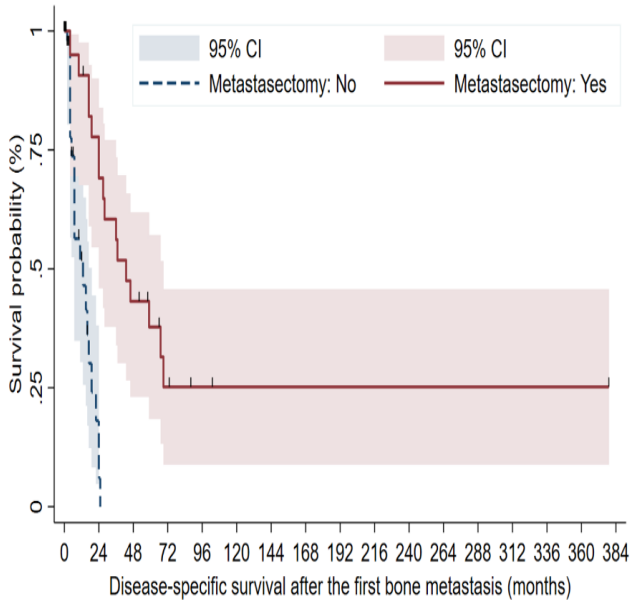
Methods: This is a single-center retrospective cohort study. From 1987- 2019, 47 patients who underwent curative-intent treatment for localized extremity bone or soft tissue sarcoma

developed a solitary bone metastasis as the first distant recurrence. The median age at the primary surgery was 46 years (Interquartile range [IQR], 35–60), and 51% (24/47) received curative-intent metastasectomy.
We compared the metastasectomy versus non-metastasectomy group characteristics and used the log-rank test and multivariate analysis to evaluate the impact of solitary bone metastasectomy. The primary outcome was overall survival after development of an isolated bone metastasis.

Results: After the first solitary bone metastasis, the metastasectomy group had longer overall survival in univariate analysis (median 43 vs. 11 months, p< 0.001; Figure 1). However, the metastasectomy group had fewer patients with metastasis in the spine or pelvis (58% vs. 96%, p=0.004) and a longer interval from primary sarcoma treatment to the identification of bone metastasis (median 28 vs. 13 months, p=0.03). In multivariate analysis, metastasectomy remained a positive prognostic factor for improved survival (HR 0.18, 95%CI 0.07 – 0.45, p< 0.001).

Conclusion: Curative-intent surgery for solitary bone metastasis from sarcoma is associated with a better prognosis and may be a reasonable treatment strategy whenever feasible. Future studies are needed to validate our findings and to investigate which patient group will benefit more from the surgery.

Figure 1. Kaplan-Meier curve of overall survival after development of a solitary isolated bone metastasis



(P 54) HIGH-THROUGHPUT DRUG SCREENING IN CHONDROSARCOMA CELLS IDENTIFIES MULTIPLE EFFICIENT ANTINEOPLASTIC AGENTS

Luyuan Li, MD, PhD[1]; Lily Hashemi[2]; Josiane E. Eid, MD[1]; Zhenfeng Duan, MD, PhD[1]; Gina Z. D'Amato, MD[3]; Ines Lohse, PhD[1]; Jonathan Trent, MD, PhD[4], (1) University of Miami, Miami, FL, (2) University of Miami, Fort Lauderdale, FL, (3) Sylvester Comprehensive Cancer Center, Miami, FL, (4) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL

Objective: Chondrosarcoma is a rare and heterogeneous group of malignant bone tumors that are characterized by the production of cartilaginous matrix. Conventional chondrosarcoma is generally resistant to traditional chemotherapy and radiotherapy. The mainstay of management for localized disease is surgical resection. Metastatic chondrosarcoma has a dismal prognosis, and to date, there are no proven effective systemic therapies. IDH mutations represent an attractive target for advanced chondrosarcoma, however, early results with IDH inhibitors in IDH-mutant chondrosarcoma are modest. As a means for drug repurposing, in this study, we aimed to identify potential therapeutic compounds for combinational therapy among FDA-approved anti-tumor agents.

Methods: We evaluated the treatment response to a panel of 215 FDA-approved anti-cancer compounds in IDH1-mutant chondrosarcoma cell lines, JJ012 and HT1080, as well as their mutant IDH1 knockout clones by means of a high-throughput drug screening platform. One thousand cells were seeded per well in 384-well micro-titer plates and treated with individual drugs over a 20,000-fold concentration range for 72 hours. Cell viability was assessed by measuring ATP levels via bioluminescence. Interpretation of dose response curve parameters was performed according to the modified drug sensitivity scoring (DSSmod) function. The drug sensitivity results were validated in clonogenic assays. The candidate drugs were further analyzed for cell death and cell cycle effects by flow cytometry.

Results: Top 20 compounds with the highest treatment efficacy were identified based on the DSSmod, and further classified according to their mechanism of action, where available. Treatment with these compounds including antimitotic (docetaxel), antimetabolite (methotrexate), HDAC inhibitor (panobinostat), anthracyclines (idarubicin and doxorubicin), topoisomerase inhibitor (camptothecin), and proteasome inhibitor (pevonedistat) for 72 hours, dramatically reduced cell viability of all tested cell lines in a dose-dependent manner, independent of IDH mutation status. The most potent representatives in each class- docetaxel, methotrexate, panobinostat, idarubicin, camptothecin, and pevonedistat- were selected and further tested for their effect on cell survival in clonogenic assays. When used within the range of their respective IC50s, the six drugs significantly reduced colony formation of the chondrosarcoma cells by 50-90%, compared with vehicle control ($p < 0.05$). Moreover, we observed that treatment with these drugs for 72 hours induced 2 to 10-fold increase in the number of chondrosarcoma cells undergoing apoptosis and/or cell cycle arrest as compared to vehicle controls ($p < 0.05$). We are currently evaluating their combinatorial antitumor effects in synergy tests.

Conclusion: We identified several highly effective agents that demonstrated anti-tumor activity in chondrosarcoma cells independent of IDH mutation status. These drugs represent attractive candidates for chondrosarcoma therapy and are worth considering for future preclinical and clinical testing.

(P 55) RELATIVE WASH-IN RATE ON DYNAMIC CONTRAST-ENHANCED MRI AS A NEW PROGNOSTIC FACTOR IN PATIENTS WITH OSTEOSARCOMA; A POSSIBLE TOOL FOR PERSONALIZED SARCOMA-CARE?

Richard E. Evenhuis, MD[1]; Gijsbert M. Kalisvaart, N/A, MD[1]; Willem Grootjans, PhD[1]; Thomas Van Den Berghe, MD[2]; Judith Bovee[3]; David Creytens, M.D.[4]; Hans Gelderblom, MD, PhD[1]; Frank Speetjens, MD, PhD[1]; Lore Lapeire, MD, PhD[5]; Gwen ML Sys, MD, PhD[2]; Marta Fiocco, PhD[1]; Koenraad Verstraete, MD, PhD[2]; Michiel van de Sande, MD, PhD[1]; Johan L. Bloem, MD, PhD[1], (1) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (2) University Hospital Ghent, Ghent, Oost-Vlaanderen, Belgium, (3) LUMC, Leiden, Zuid-Holland, Netherlands, (4) Ghent University Hospital, Gent, Oost-Vlaanderen, Belgium, (5) Ghent University, Gent, Oost-Vlaanderen, Belgium

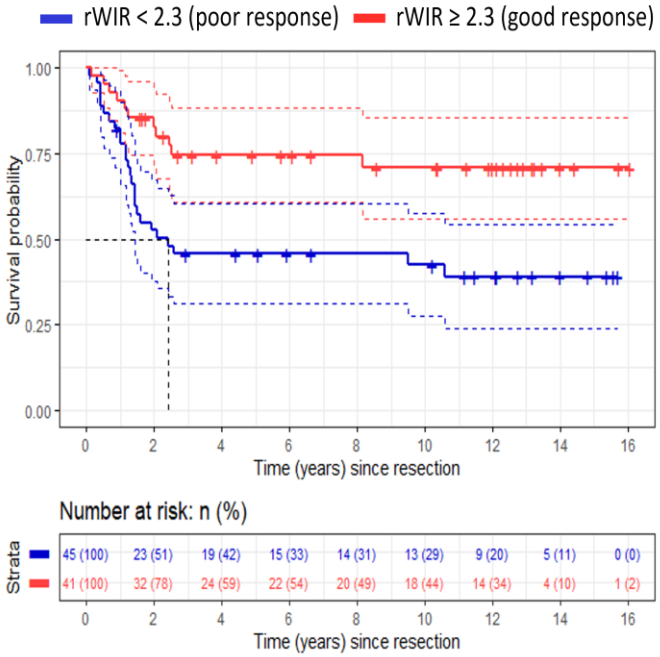
Objective: Poor histological response to neoadjuvant chemotherapy is associated with impaired prognosis in osteosarcoma. Although considered the gold standard for evaluating response to chemotherapy, histological response assessment has limitations, including sampling bias due to the examination of only one slab of tumor tissue, high inter-observer variability, and the use of a binary cut-off (≥10% viable tumor cells indicate poor response). In our previous work (to be published) we found that relative wash-in rate (rWIR) as determined by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) correlates with histological response. In this study, we aim to explore the potential of this novel radiological biomarker as prognostic factor for clinical outcome such as event-free survival (EFS).

Methods: Patients with high-grade skeletal osteosarcoma, treated with curative intent, between 2005 and 2022 at University Hospital Ghent (UHG) and Leiden University Medical Center (LUMC) were retrospectively included. All patients underwent DCE-MRI before neoadjuvant treatment and during or after receiving the second round of neoadjuvant chemotherapy, prior to surgical resection. For image analysis, the largest tumor slab was manually segmented in the DCE-MR images. The rWIR was calculated by dividing the wash-in rate on baseline imaging, defined as the maximum percentual intensity rise per second, by the wash-in rate post-chemotherapy. A rWIR < 2.3 was considered a radiological poor and rWIR ≥ 2.3 a good response based on our previous report. A previously published population study (Evenhuis et al, Cancers 2021) in 402 patients was used to identify relevant prognostic factors for EFS in Cox-proportional hazards regression modelling. EFS was defined as the time from resection to first event which consisted of local recurrence (LR), progression of metastasis, new metastasis, or death. First, multivariate Cox regression was used to estimate the effect of known clinical prognostic factors and histological response on EFS. Subsequently, this was repeated using rWIR of DCE-MRI as prognostic factor instead of histological response. EFS was estimated with Kaplan-Meier's methodology.

Results: In total, 86 patients were included with a median age of 18 years (IQR 14-31) and a median follow-up time of 11.9 years (95%CI 10.9-12.9) in which 37 events occurred. In 46 (53%) patients histological response was classified as poor while in 45 (52%) radiological response was classified as poor. In 19 patients (22%) histological and radiological classifications were discordant. Evenhuis et al. identified age group, tumor size, and metastasis at presentation as significant covariates of response for EFS estimation. When combined with histological response in Cox-regression analysis, only the presence of distant metastasis at presentation [Hazard ratio (HR) 3.58 (95%CI 1.63-7.89)], and not histological response, [HR 1.69 (95%CI 0.84-3.40)], was significantly associated with poor EFS. When using rWIR instead

of histological response as risk factor in the Cox model, the presence of distant metastasis at presentation, [HR 3.63 (95%CI 1.65-8.01)] and rWIR < 2.3, [HR 2.64 (95%CI 1.29-5.43)] were associated with poor EFS. Patients with rWIR < 2.3, had a 2-year EFS of 53% (95%CI 38-67%) vs. 83% (95%CI 71-94%) in patients with good radiological response (rWIR≥2.3).

Conclusion: The new biomarker, rWIR < 2.3, is associated with poor EFS in a multivariable analysis where the most important covariates were incorporated in a Cox model. In this cohort rWIR was associated with EFS when corrected for covariates while histological response was not. Advantages of rWIR as a response biomarker relative to histology are that it can be estimated pre-operatively instead of postoperatively and the whole tumor volume can be assessed. These findings suggest rWIR could potentially be a tool for individualized patient-care.



(P 56) CHARACTERIZATION OF DENOSUMAB TREATMENT RESPONSE ON MR PERFUSION IN GIANT CELL TUMORS OF BONE

Gijsbert M. Kalisvaart, N/A, MD; Lizz van der Heijden, MD, PhD; Ana Navas, MD; Michiel van de Sande, MD, PhD; Hans Gelderblom, MD, PhD; Kirsten van Langevelde, N/A, MD, PhD, Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands

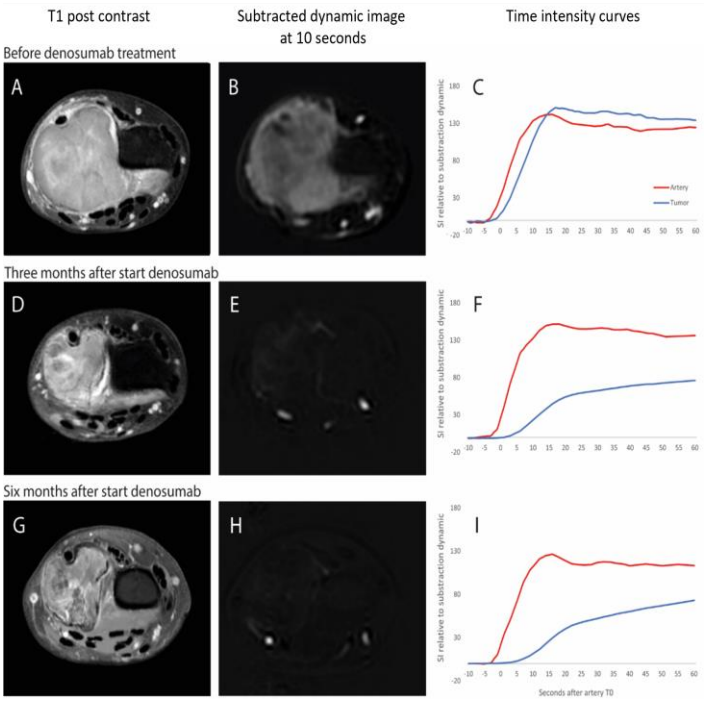
Objective: MR perfusion is useful for characterization of bone tumors, such as giant cell tumor of bone (GCTB). Denosumab is a monoclonal antibody used neo-adjuvantly in GCTB to facilitate surgery; or long term for axial tumors where surgery comes with high morbidity. We assessed changes in time intensity curve (TIC) - derived perfusion features on dynamic contrast-enhanced MR imaging (DCE-MRI) in GCTB during denosumab treatment and evaluated the duration of treatment effects that occur.

Methods: Patients with GCTB who were treated at the Leiden University Medical Center, and

underwent MRI before (t=0) and after 3 (t=3), 6 (t=6) or 12 (t=12) months of denosumab treatment (n=24), were retrospectively included. Regions of interest (ROIs) were manually placed on tumor compartments with visually most intense enhancement on DCE-MRI and intensity curves were created. Time-to-enhancement (TTE), wash-in rate (WIR), maximal relative enhancement (MRE), and area-under-the-curve (AUC) were calculated.

Results: Visual assessment of mean TICs showed relatively early and steep enhancement followed by slow wash-out of contrast before start of treatment at t=0. All 24 patients showed denosumab treatment response on MR perfusion and delay in arrival of contrast and reduced enhancement was seen within 3 months of treatment. TTE increased between t=0 and t=3 ($p< 0.001$). WIR, MRE and AUC decreased between t=0 and t=3 ($p< 0.001$, $p=0.01$ and $p=0.02$, respectively). No significant differences in features were found between t=3 and t=6 or t=6 and t=12. We found no significant perfusion differences in primary versus recurrent, or axial versus appendicular tumors.

Conclusion: MR perfusion significantly changed in GCTB within 3 months of denosumab treatment compared to baseline. No further change occurred at t=6 or t=12 suggesting that maintenance treatment with lower doses may already be indicated after 3 months. In axial tumor cases where long term denosumab is the preferred therapy, monitoring change in tumor characteristics on MR perfusion may aid optimal drug dose titration and intervals, thereby minimizing side effects.



(P 57) REAL-WORLD EXPERIENCE OF CABOZANTINIB IN PAEDIATRIC, TEENAGE AND YOUNG ADULT (TYA) AND ADULT METASTATIC BONE SARCOMA PATIENTS FROM THE LONDON SARCOMA SERVICE (LSS)

Georgina Elizabeth Wood, MBBS MRCP BSc(hons) MSc PhD[1]; Shalini Fernando, MBChB MRCP PGCert[1]; Clare Geoghegan, MPharm PGDip[1]; Denise Connolly, MD[1]; Ayesha Fathani, MBBS, MRCPCH[2]; Sandra J. Strauss, MD PhD FRCP[3]; Rachael Windsor, BSc Hons MBBS Hons MRCPCH MSc MDRes[1], (1) University College London Hospital, London, England, United Kingdom, (2) University College London Hospital, Bromley/ London, England, United Kingdom, (3) UCL Cancer Institute, London, England, United Kingdom

Objective: Sarcomas are rare, highly heterogenous solid tumours. Osteosarcoma (OS) and Ewing Sarcoma (ES) are the most common bone malignancies in children and young adults. Patients with relapsed OS and ES have a median overall survival of 12 – 18 months. Treatment options for advanced sarcoma are limited. Cabozantinib is an oral multi-kinase inhibitor (MET, VEGFR, AXL) that has demonstrated relevant pre-clinical efficacy in OS and ES. Moreover, the phase II CABONE trial demonstrated anti-tumour activity of cabozantinib in OS and ES patients with an overall response rate (ORR) of 11.9% and 19% respectively. Median progression free survival (PFS) was 6.7 months.

This study reports the real-world experience of bone sarcoma patients treated with cabozantinib within the London Sarcoma Service (LSS).

Methods: Single centre retrospective analysis of paediatric, teenage and young adult (TYA) and adult metastatic bone sarcoma patients treated with compassionate-use cabozantinib within the LSS between May 2021 and May 2023.
Patients were identified using a local electronic health record system. Patients were radiologically evaluated according to standard of care, most commonly after two or three cycles of treatment (8-12 weeks). Clinical benefit was defined as lack of clinical or radiological progression. Drug related adverse events were assessed using CTCAE version 5. Time on treatment and cabozantinib dose, including dose modification, were also assessed.

Results: Twenty-four patients were identified with a median age of 23 years (range 13-63); three paediatric patients (< 16 years), eight TYA patients (16 – 24 years) and thirteen adult patients (>25 years). Diagnoses included OS (n=16), ES (n=6) and other diagnoses (n=2) which included BCOR-CCNB3 sarcoma and mesenchymal chondrosarcoma. Median number of prior therapies was 3 (range 1 - 7). Median time from diagnosis to commencing cabozantinib was 34 weeks (range 6 - 203).

The median duration on treatment was 12.5 weeks across all ages (range 2 – 105); 8 weeks for paediatric/TYA patients and 14 weeks for adults. Eight patients remain on treatment. The most common reported drug-related grade 3 adverse events were pneumothorax (n=2), proteinuria (n=1), fatigue (n=2), rash (n=1), hypothyroidism (n=1), myalgia (n=1) and mucositis (n=1) (Figure 1).

Sixteen patients commenced treatment on 60mg daily. Five of these patients (31%) required a dose reduction to 40mg. Eight patients commenced on 40mg daily and dose escalation to 60mg was possible for three of these patients.

Eighteen patients (75%) were available for best radiological assessment at a median of 11 weeks (range 5 to 21) after starting treatment. Eight patients showed clinical benefit; one patient demonstrated a partial response, six had stable disease and one had a mixed response. Ten patients showed progressive disease. In paediatric/TYA patients the primary reason for treatment discontinuation was progressive disease (six of eleven patients). In the adult

population four of five patients stopped treatment due to toxicity and lack of clinical benefit. Median overall survival on cabozantinib was 5.3 months (range 1.6 – 26.4), with nine patients still alive (Figure 2).

Conclusion: In patients with recurrent, metastatic bone sarcomas, cabozantinib may offer benefit for select patients, but overall outcomes were poor with treatment-related toxicity that requires monitoring. Its use in combination with adjuvant chemotherapy or maintenance therapy may lead to better outcomes and should be explored within randomised clinical trials. International collaborative prospective 'real world' data registries, such as the SACHA study may provide additional insights.

Figure 1: Drug related adverse events

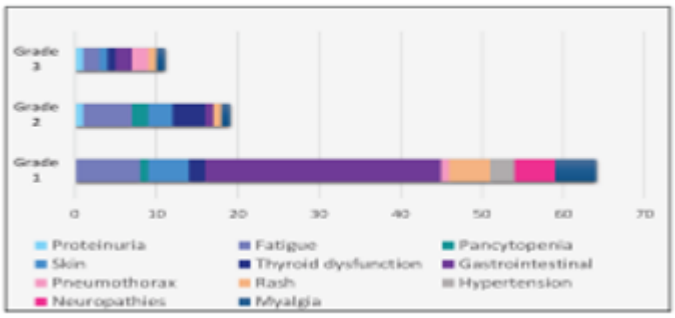
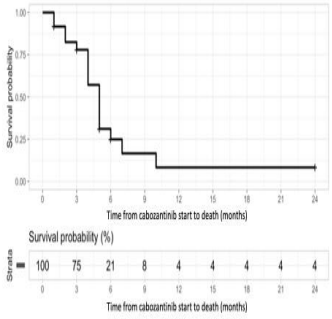


Figure 2: Kaplan-Meier curve of survival probability



(P 58) CHEMOTHERAPY-INDUCED EVOLUTION OF OSTEOSARCOMA REVEALED BY SINGLE-CELL RNA SEQUENCING

TINGTING REN, N/A, Ph.D[1]; jie zang, MD[2]; Yiyang Yu, MD[3]; Xin sun, N/A, MD[3]; Wei Guo, N/A, M.D.[3]; Xiaodong Tang, M.D.[3], (1) Peking University People's Hospital, beijing, Beijing, China (People's Republic), (2) Peking University People's Hospital, BEIJING, Beijing, China (People's Republic), (3) Peking University People's Hospital, Beijing, Beijing, China (People's Republic)

Objective: Osteosarcoma (OS) is the most common primary malignant bone tumor, particularly among children and adolescents. Neoadjuvant (preoperative) chemotherapy followed by surgery and adjuvant (postoperative) chemotherapy are the primary therapeutic strategy for OS. However, a considerable proportion of OS patients do not respond well to neoadjuvant chemotherapy and poor response stands as an obstacle to good prognosis. cancer cell intrinsic heterogeneity, cells within the tumor microenvironment (TME) and cellular plasticity were the major reasons of drug resistance. In this study, we performed scRNA-seq analyses on matched OS samples to reveal the heterogeneity of OS and cellular plasticity under drug resistance.

Methods: ScRNA-seq analyses were performed matched OS samples obtained from 5 patients before initiating systemic treatment (pre-treatment) and 3 paired surgical tumor samples after the standard neoadjuvant chemotherapy(post-treatment) which considered poor histological responders. Cell types and the corresponding marker genes were identified by Seurat and UMAP methods. Copy number Karyotyping of Tumors (copyKat) analysis was performed to identified the malignant cells. DEGs identification and GO enrichment analysis were used for differentially over-expressed genes. Tissue Microarray (TMA) and Immunohistochemical staining were used for protein validation.

Results: 13 cell clusters were identified. The proportions of endothelial cells and myofibrocytes in post-treatment samples were significantly increased compare with pre-treatment samples. While, B cells and neutrophil cells varied greatly among patients, the infiltration levels in pre-treatment samples are much higher than in post-treatment samples. Checkpoint genes (PDCD1, CTLA-4, TIGIT, LAG3 and VSIR) highly expressed in T cells of pre-non-respond samples compare with pre-respond samples, while chemotherapy did not change their expressions. Chemotherapy-induced evolution in some types of cells of post-treatment tumors were observed. The subtypes of B cells have changed after chemotherapy. IGHG B cells became the predominant cells in post-treatment samples while the other two B cells decreased sharply. Cancer-related pathways were upregulated in the post-treatment tumor cells indicated evolution of tumor cells induced by chemotherapy. Patients with more MYC+ ANGPTL4+ CPE+ HSPA8+ IFITM5+ MRPL4+ tumor cells showed a worse prognosis, which also validated by IHC staining.

Conclusion: Our results focused on the unique aspects of the chemotherapy-induced evolution in OS, that might guide the development of optimized chemotherapy to benefit poor response of OS patient.

(P 59) CHONDROSARCOMA OF THE FLAT BONES: DIFFERENTIAL SURVIVAL BETWEEN HIGH GRADE LESIONS OF THE PELVIS AND SCAPULA

Samuel Broida, N/A, MD[1]; Mikaela Sullivan, MD[2]; Emmett Cleary, MD[2]; Alexandra Arguello, MD[2]; Matthew T. Houdek, MD[2], (1) Mayo Clinic (Rochester), Rochester, MN, (2) Mayo Clinic, Rochester, MN

Objective: Studies have shown flat bone chondrosarcoma have worse outcomes than extremity tumors, but there is no data directly comparing different flat bone lesions. The primary aim of this study was to examine differences in recurrence and survival between pelvic and scapular chondrosarcoma.

Methods: Forty-two patients with scapular chondrosarcoma and 127 patients with pelvic chondrosarcoma who underwent surgical resection between 1989 and 2022 were retrospectively reviewed. Demographic and tumor characteristics were collected (Table 1) in addition to disease-specific survival (DSS) and recurrence-free survival (RFS). High grade tumors were defined as lesions that were grade 3 or 4 on surgical pathology.

Results: Patients with low-intermediate grade lesions of the scapula were more likely to have positive margins during definitive surgical management (14% vs 3%, OR 5, 95% CI [1.15,22.6], p=0.02), however this did not translate to differences in recurrence or survival (p>0.05). Among high grade tumors, patients with scapular lesions had worse RFS (HR 3.43, [1.21, 9.75], p=0.02) and DSS (HR 2.99, 95% CI [1.05, 8.51], p=0.04) (Figure 1). Five-year DSS for high grade pelvic and scapular tumors was 55% and 17%, respectively. There were no significant differences between the rate of positive margins or minimum tumor-free margin on final pathology for high grade lesions (p>0.10).

Conclusion: Five-year DSS survival for high grade chondrosarcoma of the flat bones is poor, particularly for those of the scapula. Despite a higher rate of positive margins for low-intermediate grade lesions of the scapula, there was no significant difference in survival compared to low-intermediate grades lesions of the pelvis.

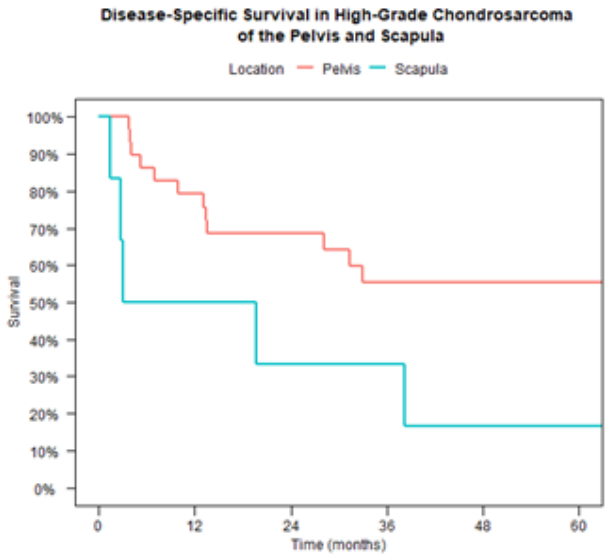


Table 1 - Demographic and Tumor Features of Patients undergoing Surgical Treatment of Pelvic and Scapular Chondrosarcoma

Characteristic	Pelvis	Scapula	Overall
Total *n*	127	42	169
Low-intermediate grade	99 (78%)	36 (86%)	135 (80%)
High grade	28 (22%)	6 (14%)	34 (20%)
Mean age at surgery, *years (range)*	52 (17-85)	46 (12-80)	51
Female	34 (27%)	14 (33%)	48 (28%)
Secondary chondrosarcoma	24 (19%)	32 (76%)	56 (33%)
Mean maximum tumor dimension, *cm*	9.6	8.1	9.4
Soft tissue mass	106 (83%)	28 (67%)	134 (79%)
Positive margins	6 (5%)	5 (12%)	11 (7%)

(P 60) SKIP LESIONS IN CHONDROSARCOMA: IS WHOLE BONE IMAGING NECESSARY?

Samuel Broida, N/A, MD[1]; Emmett Cleary, MD[2]; Mikaela Sullivan, MD[2]; Alexandra Arguello, MD[2]; Matthew T. Houdek, MD[2], (1) Mayo Clinic (Rochester), Rochester, MN, (2) Mayo Clinic, Rochester, MN

Objective: Skip metastases are rare in primary sarcoma. The utility of whole-bone imaging has not been assessed for chondrosarcoma. We aimed to describe the incidence of skip metastasis in long bone chondrosarcoma as well as the value of imaging modalities in detecting these lesions.

Methods: Medical records for patients diagnosed with chondrosarcoma of the femur, tibia/fibula, humerus, or radius/ulna between 1994 and 2022 were retrospectively obtained. Images as well as surgical pathology reports were reviewed to assess for the presence of skip metastasis.

Results: Of 135 patients with chondrosarcoma involving a long bone, five patients (3.7%) had skip lesions (Figures 1&2). All skip lesions were the same grade (3 low grade & 2 dedifferentiated) as the primary tumor. The mean distance from the primary chondrosarcoma to the skip metastasis was 1.2cm (range 0.5-2cm). Skip metastases were visible on pre-operative MRI in all 5 patients, x-ray in 1 of 5 patients, and CT scan in 3 of 3 patients. Of the 74 patients

with primary lesions confined to the metaphysis or epiphysis, 58 (78%) had whole bone imaging in the form of x-ray (n=31), MRI (n=12), or CT scan (n=10). Whole bone imaging did not detect additional lesions in any of these patients.

Conclusion: Skip metastases have a ~4% incidence in long bone chondrosarcoma. All skip lesions were ≤2cm from the primary tumor. X-rays were unreliable whereas all skip lesions were visible on MRI, though the addition of whole-bone MRI in non-diaphyseal lesions did not provide any further value. Given the rarity of skip metastasis and the above findings, the utility of whole-bone imaging is low in detection of skip lesions in chondrosarcoma of the long bones, particularly for non-diaphyseal tumors.

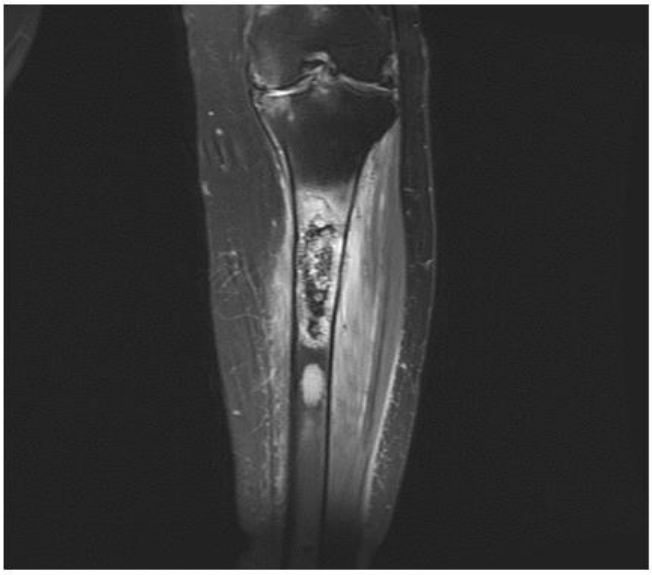


Figure 1 - Skip metastasis located 1.1cm distal to a diaphyseal chondrosarcoma of the tibia.

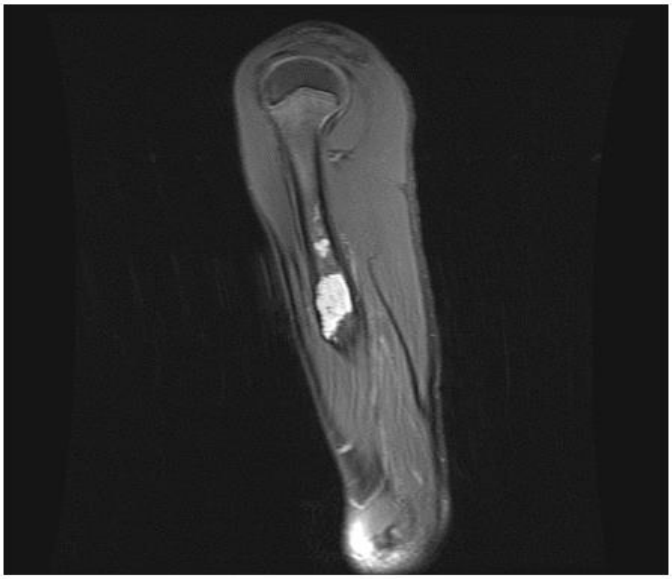


Figure 2 - Skip metastasis located 1cm proximal to a diaphyseal chondrosarcoma of the humerus.

(P 61) ADJACENT VENOUS TUMOR THROMBUS IN PRIMARY OSTEOSARCOMA OF THE PELVIS AND EXTREMITIES

Samuel Broida, N/A, MD[1]; Mikaela Sullivan, MD[2]; Alexandra Arguello, MD[2]; Steven Robinson, MBBS[2]; Scott Okuno, M.D.[2]; Thanh P. Ho, MD[2]; Brittany Siontis, MD[2]; Doris E. Wenger, MD[2]; Matthew T. Houdek, MD[2], (1) Mayo Clinic (Rochester), Rochester, MN, (2) Mayo Clinic, Rochester, MN

Objective: Despite the high rate of venous tumor thrombus (VTT) in osteosarcoma of the pelvis, there are few descriptions of VTT associated with extrapelvic primary osteosarcoma. We sought to further describe the prevalence and presenting features of venous tumor thrombus in osteosarcoma of both the pelvis and extremities.

Methods: We reviewed 308 patients with osteosarcoma treated between 2000 and 2022 at our institution. Primary lesions were located in the upper extremity (n=40), lower extremity (n=198), or pelvis (n=70). Medical records and histopathology were reviewed to identify patients with thrombi in proximity to their primary lesion.

Results: Tumors abutted vessels in 131 cases (42.5%) and encased vessels in 30 cases (9.7%). Twenty-one patients were diagnosed with VTT (Table 1). The rate of VTT was 25% for pelvic and 1.7% for extremity osteosarcoma. The most common imaging features associated with VTT were enhancement with contrast (100%), venous enlargement (83%), vessel

encasement (66%), and visible intraluminal osteoid matrix (50%). Disease-specific survival (DSS) for patients with VTT was 95% at 12 months, 49% at three years, and 31% at five years. When controlling for presence of metastatic disease, VTT was associated with worse DSS (HR 2.3, 95% CI [1.11, 4.84]) (Figure 1).

Conclusion: VTT is rare within osteosarcoma and occurs more commonly in the pelvis than the extremities. Imaging features include enhancement with contrast, venous dilation, and vessel encasement. VTT portends a worse prognosis for patients with osteosarcoma with a similar survivability to metastatic disease.

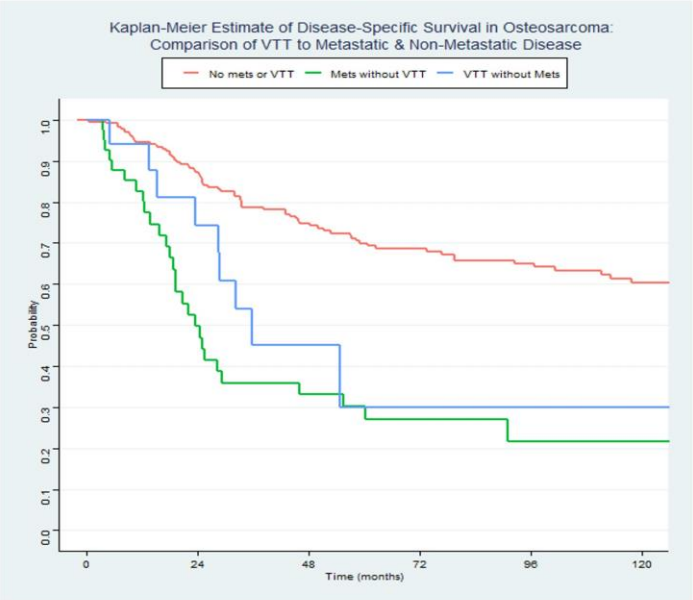


Figure 1. – Kaplan-Meier survival curves of disease-specific survival in patients with VTT without metastatic osteosarcoma compared to patients with localized or metastatic osteosarcoma without VTT

Table 1. Patients with Venous Tumor Thrombus in Osteosarcoma of the Pelvis & Extremities

No.	Age	Sex	Primary Site	Distal Extent of Tumor Thrombus	Mode of Detection	Metastases on Presentation	Chemotherapy	Local Control	Recurrence (Location, time to recurrence)	Follow Up (Status, duration of follow-up)
1	16	F	Femur	Femoral vein	Intraoperative pathology	No	Yes	Limb salvage	Local, 3 years	DOD, 55 months
2	16	F	Pelvis	External iliac vein	MRI	Yes, lung	Yes	Internal hemipelvectomy	Local, 1.5 years	DOD, 32 months
3	47	M	Pelvis	Internal iliac vein	Intraoperative pathology	No	Yes	Definitive radiation[a]		DOD, 24 months
4	16	F	Pelvis	Small vein near common iliac	Intraoperative pathology	No	Yes	External hemipelvectomy		AWD, 12 years
5	27	F	Pelvis	Internal iliac vein	CT Angiogram	No	Yes	External hemipelvectomy		AWD, 6 years
6	25	F	Femur	Femoral vein	Ultrasound	Yes, lung	Yes	Amputation		AWD, 13 years
7	48	F	Femur	Popliteal vein	MRI, CT Angiogram	No	Yes	Hip disarticulation		AWD, 4 years
8	18	F	Tibia	Common femoral vein	Intraoperative pathology	No	Yes	Hip disarticulation		AWD, 2 months
9	65	M	Pelvis	Common iliac vein	MRI	Yes, lung	Yes	Palliative radiation		DOD, 14 months
10	19	M	Pelvis	Common iliac vein	CT with contrast	No	Yes	External hemipelvectomy	Local, 4 months	DOD, 28 months
11	16	M	Pelvis	Common iliac vein	MRI	No	Yes	Definitive radiation		DOD, 47 months
12	17	M	Pelvis	Common iliac vein	MRI	No	Yes	Internal hemipelvectomy		DOD, 15 months
13	16	F	Pelvis	Internal iliac vein	MRI	No	Yes	Definitive radiation		DOD, 32 months
14	17	F	Pelvis	Internal iliac vein	MRI	No	No[b]	None		DOD, 5 months
15	23	M	Pelvis	Superior gluteal vein	MRI, CT Angiogram	No	Yes	Definitive radiation	Distant (lungs), 21 months	AWD, 34 months
16	16	M	Pelvis	Internal iliac vein	CT Angiogram	No	Yes	Total sacrectomy		AWD, 6 months
17	36	M	Pelvis	Internal iliac vein	MRI, MR Angiogram	No	Yes	External hemipelvectomy	Local, 20 months	DOD, 34 months
18	31	M	Pelvis	Internal iliac vein	CT Angiogram	No	Yes	Definitive radiation		DOD, 28 months
19	53	M	Pelvis	Internal iliac vein	MRI	No	Yes	Definitive radiation	Distant (lungs), 4 months	AWD, 14 months
20	30	F	Pelvis	Superior gluteal vein	MRI, CT Angiogram	Yes, lung	Yes	None		DOD, 36 months
21	15	M	Pelvis	Superior gluteal vein	MRI	No	Yes	Definitive radiation	Distant (lungs), 12 months	AWD, 85 months

[a]Resection aborted intraoperatively upon discovery of extensive tumor thrombus. [b]Patient died of disease prior to initiation of chemotherapy.
DOD: Died of disease; ANED: Alive with no evidence of disease; AWD: Alive with evidence of disease

(P 62) PROSTHESIS-ASSOCIATED SARCOMA: A REVIEW OF 8 HIGH-GRADE LESIONS ARISING AROUND A TOTAL JOINT ARTHROPLASTY IMPLANT

Samuel Broida, N/A, MD[1]; Mikaela Sullivan, MD[2]; Alexandra Arguello, MD[2]; Doris E. Wenger, MD[2]; Andrew Folpe, MD[2]; Matthew T. Houdek, MD[2], (1) Mayo Clinic (Rochester), Rochester, MN, (2) Mayo Clinic, Rochester, MN

Objective: Rare instances of sarcoma arising near a metallic implant have been described. We sought to present a cohort of patients who developed high-grade sarcoma around an arthroplasty prosthesis.

Methods: A retrospective record review was conducted for 8 patients who were diagnosed with sarcoma arising around a joint prosthesis. All patients were evaluated at a single institution between 2008 and 2021. Mean age of patients at sarcoma diagnosis was 65 years and 63% of patients were male. The indication for the index joint replacement was osteoarthritis in seven patients and developmental hip dysplasia in a single patient. The mean time from prosthesis implantation to sarcoma diagnosis was 10.7 years (range 4-26).

Results: All sarcomas were discovered around a lower extremity prosthesis. Two tumors were associated with a distal femur TKA prosthesis, three were associated with a proximal femoral THA stem (Figure 1), and three were located within the hip joint of patients with THA prostheses. Four lesions were detected on intraoperative pathology, three were detected on

radiographs indicated by joint pain, and one was detected via surveillance PET-CT for melanoma.
All lesions were high-grade. Seven of eight tumors had a soft tissue component in addition to osseous involvement. Histologically, 4 tumors were undifferentiated pleomorphic sarcomas, composed of markedly pleomorphic spindled cells with brisk mitotic activity and foci of necrosis; immunohistochemical studies showed a purely fibroblastic phenotype. Two angiosarcomas were seen, one of which showed predominantly epithelioid morphology and was originally confused with metastatic carcinoma. Both cases showed formation of irregular vascular channels by highly malignant-appearing endothelial cells with prominent nucleoli and easily identified mitotic figures. Markers of endothelial differentiation (e.g., CD31, ERG protein, FLI1 protein) were positive. The last two cases showed formation of osteocartilaginous matrix by pleomorphic spindled and epithelioid cells and were classified as osteosarcomas.
Four patients had developed metastatic disease (lung [n=3] and scapula [n=1] prior to treatment. Six patients underwent surgical resection in the form of limb salvage (n=3) or amputation (n=3). Five patients were treated with chemotherapy and two patients received radiotherapy. One patient developed local recurrence 10 months following limb salvage. Another patient developed lung metastases 10 months following hip disarticulation. Four patients died of disease at a mean 8.3 months (range-2-14) from diagnosis. The remaining four patients were alive at final follow-up (mean survival 28 months, range 15-57).

Conclusion: Sarcoma arising around an arthroplasty prosthesis is a rare entity and occurs years after implantation. In our series, these were most commonly discovered incidentally on intraoperative pathology or via surveillance imaging and all were high-grade lesions. Fifty-percent of patients presented with metastatic disease or developed metastases prior to initiation of treatment. Outcomes in this population are poor with half of patients succumbing to disease within 14 months.

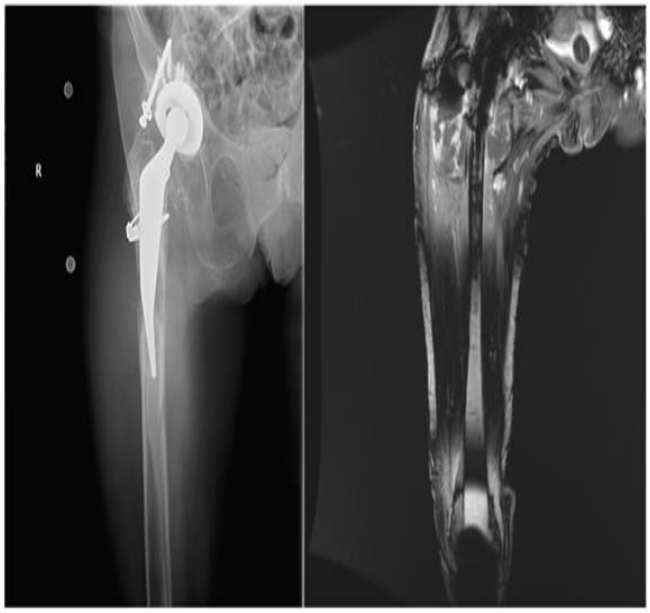


Figure 1 - AP radiograph (Left) and coronal T1 image with gadolinium (Right) of a 65-year-old female with angiosarcoma arising around a femoral prosthesis 26 years after total hip arthroplasty.

(P 63) EVALUATION OF LOCAL RECURRENCE AND DIAGNOSTIC DISCORDANCE IN CHONDROSARCOMA PATIENTS UNDERGOING PREOPERATIVE BIOPSY

Gayathri Vijayakumar, BS[1]; Luke Kasson, BS[2]; Conor Jones, MD[2]; Austin Yu, BS[3]; Linus Lee, MD[2]; Matthew Colman, MD[2]; Steven Gitelis, MD[2]; Alan T. Blank, MD, MS[2], (1) Rush University Medical Center, Edison, NJ, (2) Rush University Medical Center, Chicago, IL, (3) Rush University Medical Center, Alhambra, CA

Objective: Preoperative biopsy of chondroid lesions has demonstrated discordance between biopsy grade and surgical resection grade. Furthermore, there is evidence to suggest risk of tumor contamination during biopsy. We evaluated our large chondrosarcoma institutional cohort to compare the rates of local recurrence based on undergoing pre-surgical biopsy, as well as other tumor characteristics and disease related outcomes.

Methods: This was a retrospective review of 75 patients who underwent surgical resection for chondrosarcoma at our institution between 2005 and 2020. Outcomes included rates of local recurrence, metastasis, and overall survival.

Results: There were no significant differences in local recurrence and recurrence free survival in the cases that had undergone pre-operative biopsy. Thirteen (28.2%) patients had discordance between histologic grade on biopsy and resection. Seven of 11 patients with dedifferentiation present on final resection were not identified on biopsy. The only independent predictor of recurrence free survival and metastasis free survival was the presence of dedifferentiation on resection.

Conclusion: To our knowledge, this is the first study evaluating risk of local recurrence in the setting of pre-surgical biopsy in chondrosarcoma patients. Although pre-operative biopsy may contaminate biopsy tracts, appropriate surgical planning and final resection results in no difference in local recurrence rates in this cohort. However, discordance rates between preoperative biopsy and resected specimen must be considered while determining clinical treatment.

Table I. Demographics, Clinical and Operative characteristics

	N (%)
Total	75
Age, median (IQR)	56 (42-65)
BMI, mean (SD)	29.64 (7.32)
Sex	
Males	37 (49.3)
Females	38 (50.7)
Presurgical biopsy	46 (61.3)
Unknown grade on biopsy	6 (13.1)
Grade 1	30 (65.2)
Grade 2	7 (15.2)
Grade 3	3 (6.5)
Dedifferentiation present	5 (10.8)
Change in grade biopsy vs resection	13 (28.2)
Resection grade	
Grade 1	38 (50.7)
Grade 2	24 (32.0)
Grade 3	13 (17.3)
Dedifferentiation present	17 (22.7)
Surgical Procedure	
En Bloc or Radical Resection	63 (84)
Intralesional Excision or Curettage	12 (16)
Intraoperative Complications	2 (2.7)

IQR, interquartile range; BMI, body mass index; SD, standard deviation

Table 2. Local Recurrence and Metastasis by Preoperative Biopsy Status and Resection Grade

	Grade 1 on Resection	Grade 2 and 3 on Resection
Total Number	5	9
Local Recurrence		
Preoperative Biopsy	3 (15.7%)	6 (22.2%)
No Preoperative Biopsy	2 (10.5%)	3 (30.0%)
Total Number	0	12
Metastasis		
Preoperative Biopsy	0	10 (37.0%)
No Preoperative Biopsy	0	2 (20%)

(P 64) OSTEOSARCOMA. PATIENT-DERIVED XENOGRAFTS MODELS TO RECAPITULATE PATIENTS' CHEMORESISTANCE AND METASTATIC DISEASE

Geoffroy Danieau, PhD[1]; Marjan Khatami, PhD student[1]; Niusha Khazaei, PhD Candidate[1]; Ana Castillo Orozco, PhD Candidate[1]; Alva Annett, PhD student[1]; Takeaki Ishii, PhD[2]; Mostafa Saquib, MSc[1]; Fatima Inigo, MA[1]; Wajih Jawhar, PhD Student[1]; Qing Wu, MD[1]; Sungmi Jung, MD[3]; Robert Turcotte, MD FRCSC[4]; Livia Garzia, PhD[1], (1) Research Institute of the McGill

University Health Center, Montreal, PQ, Canada, (2) Research Institute of the McGill University Health Center, Tokyo, Tokyo, Japan, (3) Department of Pathology, McGill University Health Center, Montreal, PQ, Canada, (4) Department of Orthopaedic Surgery, McGill University Health Center, Montreal, PQ, Canada

Objective: Osteosarcoma (OS) is the most common malignant bone tumor in the pediatric and young adult's population. The introduction of neo-adjuvant polychemotherapy in the 1980s was a massive success for patients with localized OS, improving the 5-year-overall survival rate to a plateau of 70%. However, the most challenging characteristic of OS is its ability to metastasize, especially in the lungs, despite the high-dose cytotoxic chemotherapy. Metastasis at diagnosis (30% of OS) or after relapse of localized OS is the leading cause of death in OS patients. Almost no improvement has been introduced for these patients in the last three decades. There are few spontaneous metastatic models in OS, and most transplantation models (patient-derived orthotopic xenografts, PDOX) are focused on the primary disease. This paucity of human-derived relapse and metastatic models contributes to the slow advances in patient care.

Methods: We wanted to develop clinically relevant OS PDOXs from OS patients' biopsies, starting from tumor samples naïves of any treatment. The PDOXs models were engineered to express the Luciferase and then implanted orthotopically in immunocompromised mice. We applied fractionated high-dose chemotherapy to tumor-bearing mice using Doxorubicin, Cisplatin, and Methotrexate (MAP regimen). At the tumor volume cut-off of 0.5cm3, the hindlimb was amputated, and the mouse was monitored by BLI until the appearance of metastatic disease progression before being euthanized. Primary, relapses, and metastases are then profiled by next-generation sequencing at the bulk and single-cell levels.

Results: Similar to what is observed in OS patients, MAP chemotherapy significantly reduces tumor growth in our models. But we can also observe that not all the PDOXs OS tumors react the same at the treatment, some tumors respond well at first before becoming chemotherapy resistant. In both groups, monitoring these mice revealed the presence of macro-metastases in the lungs by BLI months after the implantation and amputation. Therefore, MAP chemotherapy does not entirely prevent metastatic spreading but tends to reduce the number of macroscopic metastases in the MAP arm (26%) compared to the vehicle arm (55%). MAP chemotherapy-treated mice have better overall survival and even achieve complete cure when compared to mice treated with surgery alone.

Conclusion: The OS chemotherapy resistance and metastatic dissemination can be modeled in vivo using neoadjuvant polychemotherapy in murine patient-derived orthotopic xenograft. We were able to generate local and distant relapses resistant to the MAP chemotherapy, samples that are being molecularly profiled by RNAseq (bulk and single-cell) and Whole Exome Sequencing to identify the most promising candidates' driver genes of the metastatic and chemo-resistant OS. These models, which recapitulate the OS patients' clinical history, will allow us to understand better the metastatic spreading of primary OS under chemotherapy treatment.

(P 65) **PROFILING EXTRACELLULAR VESICLES AND NANOPARTICLES IN OSTEOSARCOMA**

Marjan Khatami, PhD student[1]; Geoffroy Danieau, PhD[1]; Lata Adnani, PhD[2]; Laura Montermini, MSc[2]; Nadim Tawil, PhD[3]; Brian Meehan, BSc[1]; Janusz Rak, MD, PhD[2]; Livia Garzia, PhD[1], (1) Research Institute of the McGill University Health Center, Montreal, PQ,

Canada, (2) McGill University, Montreal, PQ, Canada, (3) McGill university, Montreal, PQ, Canada

Objective: Osteosarcoma is a malignant bone tumor primarily arising in osteoblasts, the cells responsible for bone formation. This type of cancer predominantly affects adolescents, although it also occurs in older adults. Standard treatment for osteosarcoma typically involves a combination of surgery and chemotherapy. Despite the intense chemotherapy regimens, recurrent and metastatic patients' outcomes have remained poor. Extracellular vesicles (EVs) have become highly significant in cancer research due to their essential involvement in intercellular communication and their potential as biomarkers for diagnosis and therapy. Recent discoveries of unique nanoparticles called exomeres and supermeres have broadened the repertoire of particles produced by mammalian cells and involved in long-range cell-to-cell communication. Initial work in carcinoma models has shown that these particles can significantly increase chemoresistance of tumors by the way of their oncoproteins' content.

Methods: High-grade KHOS and U2OS osteosarcoma cell lines and human fetal osteoblasts (hFOB1.19) were cultured in serum-free conditioned media for 48h. Subsequently, EVs and nanoparticles are isolated from osteosarcoma and osteoblast cell lines using a multi-step ultracentrifugation method based on size and density. Nanoparticle tracking analysis (NTA) is employed to analyze the particles' sizes; however, its ability to detect nanoparticles smaller than 30nm, such as exomeres and supermeres is limited. Transmission electron microscopy (TEM) is used to validate the particles' sizes, providing visualization and characterization of EVs and nanoparticles based on their diameters performed on gradient-purified samples, including small EVs, exomeres, and supermeres. To obtain the proteomic profile of each type of particles, we used an untargeted mass spectrometry approach.

Results: Osteosarcoma cell lines and normal osteoblasts all produce EVs of the expected sizes. Transmission electron microscopy reveals the presence of large and small EVs in their respective fractions. Importantly, TEM images of exomeres and supermeres fractions reveal for the first time the presence of particles produced by osteoblasts and osteosarcoma cells. This is the first time that non-transformed mammalian cells are shown to produce exomeres and supermeres. Previous work had focused on carcinoma-derived cell lines notorious for high-secretory activity.

Conclusion: This study presents the isolation and characterization of unique extracellular nanoparticles termed exomeres and supermeres. These nanoparticles display evident differences in terms of their size, morphology, and composition. Osteoblasts and osteosarcoma cells' secretory activity contribute majorly to the disease pathogenesis, influencing the microenvironment to promote bone destruction. Our work shows that alongside EVs and soluble cytokines, exomeres and supermeres hold the potential to function as agents of intercellular communication and can significantly impact the tumor microenvironment.

(P 66) PHASE I STUDY OF THE MUTANT IDH1 INHIBITOR IVOSIDENIB: LONG-TERM SAFETY AND CLINICAL ACTIVITY IN PATIENTS WITH CONVENTIONAL CHONDROSARCOMA

William D. Tap, MD[1]; Gregory M. Cote, MD, PhD[2]; Howard Burris, MD[3]; Lia Gore, MD[4]; Murali Beeram, MD[5]; Anthony P. Conley, MD[6]; Diego Gianolio, PhD[7]; Zhe Qu, PhD[7]; Camelia Gliser, n/a[7]; Susan Pandya, MD[8]; Jonathan Trent, MD, PhD[9], (1) Memorial Sloan Kettering Cancer Center, New York, NY, (2) Mass General Cancer Center, Boston, MA, (3) Sarah Cannon

Research Institute, Nashville, TN, (4) University of Colorado Cancer Center, Aurora, CO, (5) START Center for Cancer Care, San Antonio, TX, (6) MD Anderson Cancer Center, Houston, TX, (7) Servier Pharmaceuticals, Boston, MA, (8) Servier Pharmaceuticals, Newton, MA, (9) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL

Objective: Chondrosarcomas are rare primary bone malignancies for which there are no approved systemic therapies. 85% of chondrosarcomas are the conventional subtype. Mutations in the isocitrate dehydrogenase 1 (IDH1) gene occur in ~50% of conventional chondrosarcomas. In a phase 1 study in patients with IDH1-mutated advanced solid tumors, ivosidenib, an oral potent inhibitor of mutant IDH1, demonstrated manageable toxicity (without dose-limiting toxicities), suppression of the oncometabolite 2-hydroxyglutarate at dose levels ≥300 mg/day, and disease control in patients with conventional chondrosarcoma. We report long-term safety, tolerability and efficacy of ivosidenib in patients with conventional chondrosarcoma.

Methods: In this phase I multicenter open-label dose-escalation and expansion study, ivosidenib was administered orally (100 mg twice/day [BID] to 1200 mg once/day [QD]) in continuous 28-day cycles. Primary outcome was safety and tolerability; secondary outcomes included clinical activity (objective response rates [ORR, defined as complete response (CR) + partial response (PR)], stable disease [SD] and progression-free survival [PFS]). Adverse events (AEs) were assessed every visit and reported per the Common Terminology Criteria for Adverse Events (CTCAE version 4.03). Responses were assessed every other cycle using Response Evaluation Criteria in Solid Tumors (RECIST version 1.1).

Results: 13 patients with advanced conventional chondrosarcoma were included in this analysis (data cut off 15 September 2022; women, n=4; median age 54.0; AJCC tumor grade I [n=2], II [n=8], III [n=1], unknown [n=2]; 6 had received prior systemic therapy; received 100 mg ivosidenib BID [n=1], 400 mg QD [n=1], 500 mg QD [n=7], 800 mg QD [n=2], and 1200 mg QD [n=2]). Median treatment duration was 11.3 months (range: 0.5-92.6 months). Four patients (30.8%) have continued therapy for >6 years (two of which were treated for >7 years). Median relative dose intensity was 100%. The most frequent treatment emergent AEs (in >4 pts; mostly grade 1/2) were diarrhea (n=5) and nausea (n=5). Six patients experienced grade ≥3 AEs. Three patients experienced serious AEs (none considered related to treatment). There were no discontinuations, dose reductions or deaths due to AEs. The ORR was 23.1%. Median duration of response was 42.5 months (range: 25.8-51.8 months). One patient with a base of the skull lesion achieved a CR (1200 mg IVO QD); two achieved PR (one 500 mg and one 1200 mg IVO QD); seven had SD (five received ≥500 mg IVO QD) and two had PD (both received ≥500 mg IVO QD). All responses occurred after >2 years on treatment. Median PFS was 7.4 months (95% CI: 2.0-61.3).

Conclusion: In patients with IDH1 mutated advanced conventional chondrosarcoma, ivosidenib demonstrates manageable toxicity and durable disease control including long-term responses, extending several years for a proportion of patients. Future studies are warranted to better understand the efficacy of ivosidenib in patients with advanced conventional chondrosarcoma.

(P 67) GENOMIC CHARACTERIZATION OF CHONDROSARCOMA: A RARE SARCOMA WITH CLINICAL RESPONSES TO IMMUNE CHECKPOINT INHIBITION

Michael J. Wagner, MD[1]; Nathan W. Sweeney, PhD[2]; Elizabeth T. T. Loggers, MD, PhD[3]; Jesse Roberts, M.D.[4]; Elyse J. Brinkmann, MD[4]; Eleanor Chen, MD, PhD[4]; Robert W. Ricciotti, MD[4]; Rachel Berg, MS, APRN, FNP-C, AOCNP[5]; Melissa Stoppler, MD[2]; Lee D. Cranmer, MD, PhD, F.A.C.P.[6], (1) Dana Farber Cancer Institute , Boston, MA, (2) Tempus Labs, Inc, Chicago, IL, (3) Fred Hutch Cancer Center, Seattle, WA, (4) University of Washington, Seattle, WA, (5) Tempus Labs, Inc, Farmington, UT, (6) University of Washington/Fred Hutchinson Cancer Center, Seattle, WA

Objective: Chondrosarcomas are rare cancers of cartilage. Conventional chondrosarcoma, the most common subtype, has no known effective systemic therapies. Mesenchymal and dedifferentiated chondrosarcoma are treated with Ewing sarcoma or osteosarcoma regimens, although the efficacy of this approach is not firmly established. More effective systemic therapies are urgently needed. After the identification of IDH mutations in chondrosarcoma, IDH inhibitors are now being studied in this disease. Reports of patients responding to immune checkpoint inhibitors are published, but without a clear mechanism for why some chondrosarcoma patients respond to immunotherapy. Small prospective studies of immune checkpoint inhibitors were negative. We sought to characterize the molecular and immune landscape of conventional, dedifferentiated, and mesenchymal chondrosarcomas using a large database of clinical-grade sequencing results.

Methods: De-identified records from patients with a histologic diagnosis of conventional chondrosarcoma, dedifferentiated chondrosarcoma, or mesenchymal chondrosarcoma in the Tempus database who had completed tissue DNA sequencing with the Tempus xT assay were included for this retrospective analysis (Tempus Labs, Chicago, IL). Tempus xT is a targeted, tumor/normal-matched panel that detects single-nucleotide variants, insertions and/or deletions, and copy number variants in 648 genes, as well as chromosomal rearrangements in 22 genes with high sensitivity and specificity. Microsatellite instability (MSI) and tumor mutational burden (TMB) were determined from sequencing data. Expression of PD-L1 and mismatch repair enzymes were evaluated in cases with available immunohistochemistry (IHC) data.

Results: There were 149 patient records identified consisting of 103 conventional chondrosarcoma, 31 dedifferentiated chondrosarcoma, and 15 mesenchymal chondrosarcoma samples. Out of the total cohort, 44% of patients had either an IDH1 or IDH2 mutation (n=65); there were no cases of co-occurring IDH1/2 mutations. No cases were MSI high. One conventional chondrosarcoma patient had a TMB >10 mut/Mb. Among 86 patients with available PD-L1 IHC data, 4% of conventional (n=4), 13% of dedifferentiated (n=4), and 13% of mesenchymal cases (n=2) were PD-L1 positive. The most common somatic alterations in conventional chondrosarcoma were IDH1 (34%), TP53 (30%), CDKN2A (18%), TERT (15%), CDKN2B (14%), LRP1B (13%), MTAP (12%), and KMT2D (11%). The most common somatic alterations in dedifferentiated chondrosarcoma were TERT (71%), TP53 (68%), IDH2 (39%), CDKN2A (39%), IDH1 (39%), CDKN2B (35%), MTAP (19%), and PDGFRB (19%). The most common somatic alterations in mesenchymal chondrosarcoma were HEY1-NCOA2 fusions (87%), KMT2C (20%), CDKN2A (20%), EMSY (13%), PRKDC (13%), RBM10 (13%), CDKN2B (13%), and MTAP (13%).

Conclusion: These findings reinforce current therapeutic efforts to target IDH signaling in chondrosarcoma and provide insight into why some subpopulations of patients may respond to immune checkpoint inhibitors. Biomarker-driven trials are needed to understand the significance and potential applications of these results in clinical practice.

(P 68) METASTASIS-DIRECTED RADIOTHERAPY FOR EWING SARCOMA PATIENTS ENROLLED ON AEWS1221: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP (COG)

Safia K. Ahmed, MD[1]; Mark Krailo, PhD[2]; Allen B. Buxton, MS[3]; Christine Hill-Kayser, MD[4]; Bree R. Eaton, MD[5]; Danny J. Indelicato, MD[6]; Sandy Kessel, BS[7]; Julia L. Glade Bender, MD[8]; Damon Reed, MD[9]; Richard Gorlick, MD[10]; Steven G. Dubois, MD, MS[11]; Nadia Laack, MD, MS[12], (1) Mayo Clinic Arizona, Phoenix, AZ, (2) University of Southern California, Los Angeles, CA, (3) Public Health Institute, Children's Oncology Group, Monrovia, CA, (4) Penn Radiation Oncology, Philadelphia, PA, (5) Emory University, Atlanta, GA, (6) UFPTI, Jacksonville, FL, (7) IROC QARC, Lincoln, RI, (8) Memorial Sloan Kettering Cancer Center, New York, NY, (9) Moffitt Cancer Center, Tampa, FL, (10) The University of Texas MD Anderson Cancer Center, Houston, TX, (11) Dana-Farber/Boston Children’s Cancer and Blood Disorders Center, Boston, MA, (12) Mayo Clinic, Rochester, MN

Objective: Local control outcomes at metastatic sites in Ewing sarcoma (ES) are largely unknown. AEWS1221 was the first COG study to prospectively evaluate stereotactic body radiation therapy (SBRT) for the treatment of bone metastases in addition to conventionally fractionated radiotherapy (CFRT). Data collection and review for all metastases-directed local therapy is ongoing. We report the available data and analysis to date regarding the approach to definitive local radiation therapy for the treatment of metastases in ES patients enrolled on AEWS1221.

Methods: AEWS1221 was a prospective, randomized trial designed to evaluate IGF-1R inhibition in newly diagnosed metastatic ES patients, with similar outcomes in both randomized arms. Definitive local therapy, defined as surgery, SBRT, or CFRT, was recommended at the end of consolidation chemotherapy for all metastatic sites documented at time of diagnosis regardless of arm assignment. SBRT was recommended for unresected bone metastases < 5 cm in size at diagnosis. CFRT was recommended for unresected metastases >5 cm at diagnosis. Whole lung irradiation (WLI) with anterior-posterior, posterior-anterior opposed fields was recommended for lung metastases. Prescription doses were: SBRT, 40 Gy in 5 fractions; CFRT, 55.8 Gy in 31 fractions; WLI, 15 Gy in 10 fractions for patients >6 years and 12 Gy in 8 fractions for patients ≤6 years.

Results: Of 298 eligible enrolled patients, 169 patients (56.7%) completed consolidation chemotherapy without relapse and had discrete metastasis at enrollment beyond bone marrow disease. Eighty-five patients (50.3%) had bone metastases at enrollment. As of May 1, 2023, 165 patients had metastases directed local therapy records submitted for central review. Eighty-one patients received WLI only (49%), 36 patients received bone metastases radiotherapy only (22%), and 20 received WLI and bone metastases radiotherapy (12%). The remaining 28 patients (17%) received radiation therapy for non-bone and lung metastases, including soft tissue metastases and distant lymph node disease. Seventy-four patients (43.5%) had bone metastases eligible for SBRT; of these, 28 (38%) received SBRT. The three-year cumulative incidence of failure at bone sites treated with SBRT was 8.4% (95% CI, 3.6-19.0%) and 20.1% (95% CI, 11.9-32.6%) at bone sites not treated with SBRT.

Conclusion: Radiation to metastatic sites in ES appears feasible as most patients enrolled on AEWS1221 received at least some form of definitive local radiation therapy to metastatic sites at end of planned chemotherapy. Preliminary review of radiation approaches demonstrate a modest percentage of eligible patients (38%) treated with SBRT, and a nominally lower

cumulative incidence of failure at bone sites treated with SBRT (8.4%) compared to bone sites not treated with SBRT (20.1%). Future analyses will evaluate local failure rates at metastatic sites by treatment modality, survival outcomes, protocol deviations, and toxicities with these approaches.

(P 69) DEVELOPMENT AND MISSION OF THE HARMONIZATION INTERNATIONAL BONE SARCOMA CONSORTIUM (HIBISCUS)

Elyssa Rubin, MD[1]; Mark Krailo, PhD[2]; Marie-Cecile Le Deley, MD, PhD[3]; Samuel Volchenboum, MD, PhD, MS[4]; Kathryn Bouzein, MS[4]; Suzi Birz, MSc[4]; Mei Li, MS[4]; Michael Watkins, PhD[4]; Damon Reed, MD[5]; Matias de Albert, MD[6]; Daniel Baumhoer, MD[7]; Stefan S. Bielack, MD, PhD[8]; Bernadette Brennan, BSC MBChB MD[9]; Line Claude, MD[10]; Uta Dirksen, MD[11]; Franca Fagioli, MD[12]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[13]; Raimondo Piana, MD[14]; Sandra J. Strauss, MD PhD FRCP[15]; Maud Toulmonde, N/A, MD[16]; Claudia Valverde, MD[17]; Milena Villaroel, MD[18]; Olga Zaikova, MD[19]; Nathalie Gaspar, PhD MD[20]; Stefanie Hecker-Nolting, MD[21]; Katherine A. Janeway, MD[22], (1) Children's Hospital of Orange County, Orange, CA, (2) University of Southern California, Los Angeles, CA, (3) Centre Oscar Lambret, Lille, Nord-Pas-de-Calais, France, (4) University of Chicago, Chicago, IL, (5) Moffitt Cancer Center, Tampa, FL, (6) Hospital Valle de Hebron, Barcelona, Catalonia, Spain, (7) University Hospital Basel, Basel, Basel-Stadt, Switzerland, (8) Olgahospital Stuttgart, Stuttgart, Baden-Wurttemberg, Germany, (9) Royal Manchester Children's Hospital, Manchester, England, United Kingdom, (10) Leon Berard Center, Lyon, Rhone-Alpes, France, (11) University Hospital Essen, Pediatrics III, West German Cancer Center, German Cancer Research Center (DKTK) site Essen, National Center for Tumordiseases (NCT) site Essen, ,, Essen, Nordrhein-Westfalen, Germany, (12) Pediatric Oncohematology Division, Regina Margherita Children's Hospital, Azienda Ospedaliera-Universitaria Città della Salute e della Scienza, University of Torino, Turin, Piemonte, Italy, (13) Royal National Orthopaedic Hospital, London, England, United Kingdom, (14) Azienda Ospedaliera Citta della Salute e della Scienza di Torino, Turin, Piemonte, Italy, (15) UCL Cancer Institute, London, England, United Kingdom, (16) Institut Bergonie, Bordeaux, Aquitaine, France, (17) Vall d'Hebron University Hospital, Barcelona, Catalonia, Spain, (18) Grupo Latinoamericano Oncología Pediátrica, Santiago, Region Metropolitana, Chile, (19) Oslo University Hospital, Oslo, Oslo, Norway, (20) Gustave Roussy cancer campus, Paris, Ile-de-France, France, (21) Klinikum der Landeshauptstadt Stuttgart - Olgahospital, Stuttgart, Baden-Wurttemberg, Germany, (22) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA

Objective: Osteosarcoma and Ewing sarcoma are rare malignancies with only about 3 cases/million/year and 1.5 cases/million/year, respectively (1,2). International efforts to aggregate data in other rare cancers has facilitated refinement of risk stratification and study of rare subtypes. We established the Harmonization International Bone Sarcoma Consortium (HIBiSCus) in May of 2019. The goal of HIBiSCus is to increase understanding and accelerate progress through larger analytic patient populations in osteosarcoma and Ewing Sarcoma. This report describes the establishment of this new data resource for the field.

Methods: The founding members of HIBiSCus partnered with the Pediatric Cancer Data Commons group, now called Data for the Common Good, at the University of Chicago who had

experience and expertise in launching consortia for other disease groups and facilitating data dictionary work (3, 4, 5, 6). A memorandum of understanding governs the Consortium. Consortium decisions are managed by an executive committee consisting of one member from each group and representatives from multiple disciplines including pathology, radiation oncology, radiology, surgery, and biostatistics. The executive committee has three Co-Chairs (Figure 1). Data dictionaries are created by assimilation, clarification, and balloting of clinical trial case report forms. Data are shared under data sharing agreements and will be accessed through a data portal by project proposal application to and approval from the executive committee.

Results: There are ten cooperative group members participating in HIBiSCus, including Children's Oncology Group (COG), Euro Ewing Consortium (EEC), National Cancer Research Institute Sarcoma Clinical Studies Group (NCRI), Cooperative German-Austrian-Swiss Osteosarcoma Study Group (COSS) of the German Pediatric Oncology-Hematology Society (GPOH), Cooperative Ewing Sarcoma Study Group (CESS) of the GPOH, French Bone Sarcoma Group GROUPOS (SFCE, GSF-GETO, UNICANCER), Spanish Sarcoma Group (GEIS), Italian Sarcoma Group (ISG), Scandinavian Sarcoma Group (SSG), and Grupo Latino Americano Oncología Pediátrica (GALOP). The memorandum of understanding was signed on April 23, 2021. Two data dictionaries have been finalized by assessing prior clinical trials and working group discussion. Data dictionaries are available at: https://commons.cri.uchicago.edu/data-dictionaries/. One data contributor agreement is signed and another nine are in progress. Finalizing data transfer agreements is anticipated to take time, especially since it includes international partnership in the context of varying regulations regarding use and sharing of patient data for research (7). Data from the first cooperative group are expected to be available in late fall 2023. Although data import is still pending, it is projected that there will be over 9500 Ewing sarcoma patients and 6800 Osteosarcoma patients in the database (Figure 2). Multidisciplinary workgroups have been devised to continue the momentum of the HIBiSCus consortium formation while awaiting data, maximize productivity as well as anticipate and address issues that arise during data harmonization. The first workgroup to be launched is the bone tumor clinical trial standards workgroup focused on guidelines for molecular assessments, staging and risk classification, data collection, and response criteria for clinical trials.

Conclusion: With our new international partnerships, HIBiSCus provides a platform to access data for research. It also provides for wider global discussions and consensus, with data expected to be deposited over the next years.

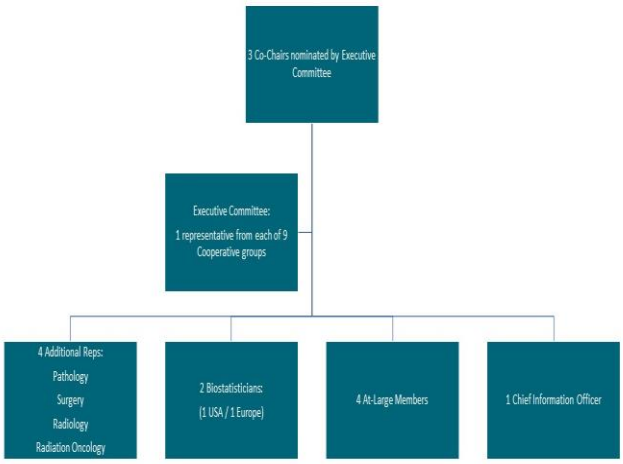


Figure 1: HIBiSCus Executive Committee Structure: Each cooperative group has one representative on the Executive Committee from which three Co-Chairs were elected. In addition, there is representation from Pathology, Radiation Oncology, Radiology, and as well as two Biostatisticians, four At-Large Members, and one Chief Information Officer

Cooperative Group*	Estimated # of Ewing sarcoma patients
COG	2584
GPOH-CESS	3581
ISG	814
GEIS	43
GALOP	531
FSG	1135
UK	866
Total	9554 patients

*Missing SSG estimates

Cooperative Group*	Estimated # of osteosarcoma patients
COG	2313
GPOH-COSS	2299
ISG	795
GEIS	69
GALOP	680
FSG	653
Total	6809 patients

*Missing UK and SSG estimates

Figure 2: Bone Sarcoma Clinical Database Patient Number Projections

(P 70) ICONIC (IMPROVING OUTCOMES THROUGH COLLABORATION IN OSTEOSARCOMA): A PROSPECTIVE OBSERVATIONAL COHORT STUDY FOR NEWLY DIAGNOSED OSTEOSARCOMA PATIENTS IN THE UNITED KINGDOM (UK)

Sandra J. Strauss, MD PhD FRCP[1]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[2]; Bernadette Brennan, BSC MBChB MD[3]; Robin Young, MBChB MRCP PhD[4]; Kenneth Rankin, FRCS[5]; Michael Parry, FRCS[6]; Jonathan D. Stevenson, MBChB FRCS (Tr&Orth)[7]; Adrienne Margaret Flanagan, MD-PhD[8]; Rachel Taylor, PhD[9]; Lorna Anne Fern, PhD[10]; Rebecca Baldwin, MSci[2]; Anna K. Tollit, MSc[11]; Filipa Vance, PhD[12]; Sharon Forsyth, BSc[13]; Kate P. Sparksman, BSc(Hons) MSc(R) RVN[14]; Krystyna Reczko, BSc[13]; William Wilson, MSc[15], (1) UCL Cancer Institute, London, England, United Kingdom, (2) Royal National Orthopaedic Hospital, London, England, United Kingdom, (3) Royal Manchester Children's Hospital, Manchester, England, United Kingdom, (4) Weston Park Cancer Centre, Sheffield, England, United Kingdom, (5) Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, England, United Kingdom, (6) Royal Orthopaedic Hospital, BIrmingham, England, United Kingdom, (7) Royal Orthopaedic Hospital, Birmingham, England, United Kingdom, (8) University College London, Cancer Institute, London, England, United Kingdom, (9) University College London Hospitals NHS Trust, London, England, United Kingdom, (10) University College London Hospitals NHS Trust, London, England, United Kingdom, (11) Royal National Orthopaedic Hospital NHS, London, England, United Kingdom, (12) University of Bath,, Bath, England, United Kingdom, (13) CR UK & UCL Cancer Trials Centre, London, England, United Kingdom, (14) Cancer Research UK & UCL Cancer Trials Centre, London, England, United Kingdom, (15) CRUK & UCL Cancer Trials Centre, London, England, United Kingdom

Objective: Osteosarcoma (OS) is a rare cancer which has seen few changes to its standard of care treatment over the last 25 years. There is an unmet need for novel therapeutic approaches if outcomes are to improve. Understanding variations in current management of the disease and identifying new prognostic and predictive markers are vital steps towards achieving this goal.

Methods: ICONIC (NCT04132895) is a multicentre prospective observational cohort study initiated to establish a platform for recruitment of newly diagnosed OS patients across the UK with matched longitudinal collection of clinical and biological samples along with patient-reported outcomes. Key objectives are to describe patient characteristics and variations in treatment across the country and correlate biological and clinical data with patient outcomes to develop prognostic biomarkers and inform development of future clinical trials, as well as better understand patient experience. The study opened to recruitment in October 2019. Here we present preliminary data for patients recruited up to February 2023.

Results: There have been 219 patients recruited to date from 24 UK sites, median age 19 (range 3-83), 57% male (Table 1). Femur (50%, 108/215), tibia/fibula (24%, 51/215) and humerus (9% 19/215) the most common anatomical sites, though less usual sites are also represented including craniofacial disease (7%, 14/215), pelvis/sacrum (5% 10/215) and rib/chest wall disease (3%, 6/215). Treatment data from 151 patients demonstrated 129 (85%) patients received a methotrexate-containing neo-adjuvant regimen. A total of 166 patients have undergone surgery, of which 36 (22%) were amputations. Multi-agent adjuvant chemotherapy treatment has been initiated in 133 patients, 99 (74%) of which included methotrexate and 50/78 (64%) of patients eligible to receive adjuvant mifamurtide following NICE recommendations have received at least 1 dose (median 27, range 1-49). A total of 41 patients have relapsed/recurrent disease during follow up (10 local, 22 metastatic, 9 both) and there

have been 34 deaths to date. With a median follow-up of 12.1 months, 1-year PFS and OS rates are 74.2% (95% CI 66.1%-80.6%) and 86.9% (95% CI 80.1%-91.5%) respectively (Figure 1). Baseline blood for germline analysis is available from 186 pts (85%), and ctDNA in 81 patients (83%). Blood samples were also received for a pilot study to identify CTC in 78 patients. We have agreement to access archival FFPE from diagnostic, and resection tissue samples as well as relapsed samples if a biopsy is performed and frozen specimens have been collected from 128 patients). PROs have been collected from 200 patients. Funding from the Bone Cancer Research Trust has been secured to extend recruitment and follow up for an additional 2 years; to perform comprehensive spatial transcriptomic and genetic analysis of longitudinal tissue samples and to develop tumour models to identify novel targets, as well as establishing an imaging repository to facilitate imaging biomarker studies and validating PROs within this cohort.

Conclusion: ICONIC is the first prospective observational cohort study to recruit UK patients of all ages with newly diagnosed OS. Initial analyses have demonstrated the feasibility of comprehensive data and sample collection and identified differences in systemic management of patients across the country worthy of further investigation ICONIC provides a unique resource that will allow for better understanding of the biology of OS and aid development of new biomarkers and novel therapeutic approaches. Analyses are aligned with objectives within the newly formed European consortium, Fight Osteosarcoma Through European Research (FOSTER) where possible and provide opportunities for wider collaboration.

(P 71) CHEMOTHERAPY AND PROGNOSTIC SIGNIFICANCE OF EXTENT OF DEDIFFERENTIATION IN DEDIFFERENTIATED PAROSTEAL OSTEOSARCOMA

Andy Livingston, MD, MS[1]; Jeffrey M. Cloutier, MD, PhD[2]; Anthony P. Conley, MD[2]; Richard Gorlick, MD[1]; Robert Benjamin, MD[1]; Kristi Posey, PA[2]; Elise F. Nassif, MD[3]; Maria A. Zarzour, MD[2]; Dejka M. Araujo, MD[1]; Ravin Ratan, MD[1]; Vinod Ravi, MD, MS, MBA[1]; Neeta Somaiah, MD[1]; Heather Lin, PhD[2]; Cheuk Hong Leung, MS[2]; Alexander J. Lazar, MD, PhD[1]; Valerae Lewis, MD[1]; Patrick Lin, MD[1]; Behrang Amini, M.D, PhD[4]; Raul F. Valenzuela, M.D[2]; Shreyaskumar Patel, MD[1]; Wei-Lien Wang, M.D[1], (1) The University of Texas MD Anderson Cancer Center, Houston, TX, (2) MD Anderson Cancer Center, Houston, TX, (3) University of Texas MD Anderson Cancer Center, Houston, TX, (4) MD Anderson cancer Center, Houston, TX

Objective: Dedifferentiated parosteal osteosarcoma (DDPOS) is a rare variant of osteosarcoma representing high-grade transformation of conventional low-grade parosteal osteosarcoma (CPOS). DDPOS is often treated similarly to other high-grade osteosarcomas; however, the impact of extent of dedifferentiation, the presence of chondroblastic heterologous differentiation, and the pathologic response to neoadjuvant chemotherapy on survival outcomes is not well-defined. We sought to evaluate the prognostic significance of the percentage and morphology of the dedifferentiated component as well as the tumor necrosis as it relates to chemotherapy benefit and clinical outcomes.

Methods: Patients diagnosed with CPOS and DDPOS between 1985-2021 were identified by retrospective review of the institutional pathology database. Clinical and treatment data were collected. Percent, morphology of dedifferentiation, and chondroblastic heterologous differentiation was assessed in resection specimens based on review of available H&E slides

by a bone and soft tissue pathologist. The Kaplan-Meier method was used to estimate rates of both overall survival (OS) and relapse-free survival (RFS). Log-rank tests were used to assess for significance of differences between groups.

Results: 71 patients with POS were identified including CPOS (n=31) and DDPOS (n=40). For DDPOS, median age at diagnosis was 36.5 years (range, 18-79). Tumors most commonly involved the femur (n=26). The majority (n=35, 88%) presented with localized disease at diagnosis. 17 patients underwent up-front resection with DDPOS identified at time of resection. Tumor size ranged from 2.4-25cm and contained varying extent of dedifferentiated component (range 5-95%). 29 of 35 patients with localized disease received chemotherapy for DDPOS. The most common neoadjuvant regimen was doxorubicin/cisplatin. Pathologic tumor necrosis was variable (range 0-95%); 3 of 23 patients exhibited >90% necrosis. DDPOS was associated with increased risk of relapse (HR 2.8 (95% CI: 1.1, 7.2), p=0.033) and of death (HR 5.1 (95% CI: 1.2, 23.0), p=0.032) as compared to CPOS. Within DDPOS, patients with metastasis at diagnosis had inferior OS (mOS 3.4 years v not reached, p=0.01). The risk of relapse/death amongst patients with DDPOS who did not receive either neoadjuvant or adjuvant chemotherapy was significantly greater than those who received chemotherapy as part of their initial treatment (HR 3.6, p=0.046). There was no difference in RFS or OS between patients stratified by % of dedifferentiation nor by exhibiting a chondroblastic component. There was no correlation between pathologic treatment response and survival outcomes in DDPOS.

Conclusion: Presence of dedifferentiation, regardless of extent, is associated with worse survival outcomes in patients with parosteal osteosarcoma. Histological response was variable and not prognostic in our limited data set. Chemotherapy should be considered as standard treatment for all patients with DDPOS.

(P 72) CO-EXPRESSION OF LEUKOCYTE CYTOSOLIC PROTEIN 1 AND MATRIX METALLOPROTEINASES ARE INTEGRAL FOR METASTATIC PROGRESSION OF CHONDROSARCOMA

Alexandra Krez, BA[1]; Nicholas Guardino, BS[2]; Caleb A. Watson, BS[2]; Emily M. Peairs, BS[3]; Makoto Nakagawa, MD, PhD[2]; Jason A. Somarelli, Ph.D[2]; Benjamin Alman, MD[2]; Julia Visgauss, MD[2], (1) Duke University School of Medicine, Scarsdale, NY, (2) Duke University, Durham, NC, (3) Duke University School of Medicine, Durham, NC

Objective: As chondrosarcoma (CSA) lacks effective systemic therapies for metastatic disease, identifying novel targets remains necessary. Our previous work identified overexpression of Leukocyte Cytosolic Protein 1 (LCP1) in metastasizing CSAs and that increased expression of LCP1 directly increases migration and invasiveness in CSA. Further, as LCP1 has been shown to correlate with matrix metalloproteinases activity and extracellular matrix degradation in non-CSA tumors, we therefore hypothesize that LCP1 with MMPs are integral to the metastatic potential of CSA.

Methods: Four primary CSA tumors were used in this study, two from metastasizing tumors (725, 731) and two from non-metastasizing tumors (732, 743). Previous studies confirmed high native expression of LCP1 in the metastasizing tumors and low expression in the non-metastasizing tumors. Their conditional knockout and overexpression conditions were generated and confirmed via western blotting. Bulk RNA sequencing data and protein quantification via Luminex assay were performed to measure expression of MMP-1 and MMP-

9. Hydrogel matrices were generated to recapitulate a 3D environment. Protein localization was demonstrated with immunocytochemistry.

Results: Bulk RNA sequencing with comparison of non-metastasizing and metastasizing cell lines, as expressed by Log 2-fold change, revealed overexpression of MMP-1 ($p< 0.001$) and MMP-9 ($p< 0.01$) in LCP1 high metastasizing tumors. Excreted MMP-1 and MMP-9 protein expression from 3D hydrogel matrices were significantly higher in the LCP1 high metastasizing compared to the LCP1 low non-metastasizing cell lines. Immunocytochemistry of the 3D hydrogel matrices demonstrated co-localization of MMPs at the invadopodia edge in LCP1 high metastasizing cell lines.

Conclusion: Our findings suggest that while LCP1 directly contributes to cell motility and invasiveness in CSA, this effect may be further mediated by increased co-expression of MMP-1 and MMP-9. Our findings suggest common regulatory machinery for the expression of LCP1 , MMP-1 and MMP-9, as well as potential for targeted agents with multiple therapeutic effects that we continue to investigate.

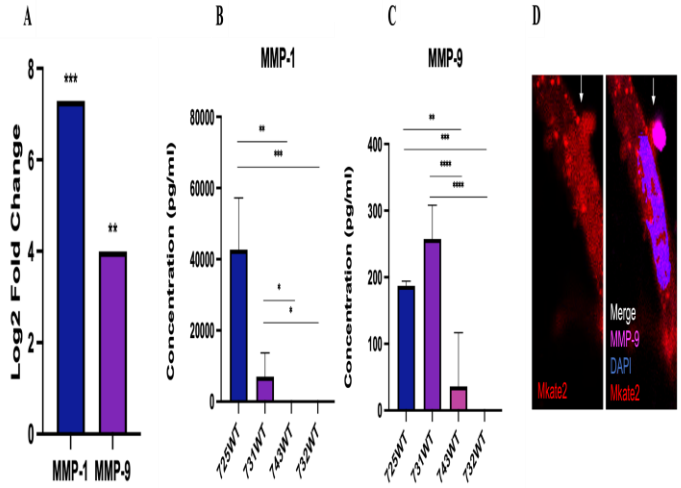


Figure 1: MMP-1 and MM-9 expression in CSA. (**A**) Differential expression of MMP-1 and MMP-9 in CSA metastatic vs. non-metastatic primary tumors via RNA sequencing (**B**) MMP-1 and (**C**) MMP-9 excreted protein expression in metastatic lines (725, 731) and non-metastatic lines (732, 743) via Luminex assay. (**D**) Fluorescent Immunocytochemistry of the 3D hydrogel matrix. White arrows localizing invadopodia and co-staining of MMP-9 [Red; Mkate2 (endogenous fluorophores), Blue; DAPI, Magenta; MMP-9].

(P 73) **FOSTER CONSORTIUM, FIGHT OSTEOSARCOMA THROUGH EUROPEAN RESEARCH**

Nathalie Gaspar, PhD MD[1]; Natalia Fernandez, PhD[2]; Pan Pantziarka, PhD[3]; Fredrik Baecklund, MD, PhD[4]; Stefan S. Bielack, MD, PhD[5]; Kjetil Boye, MD, PhD[6]; Michael Capra, MD[7]; Marco Gambarotti, MD[8]; Hans Gelderblom, MD, PhD[9]; Leo Kager, MD[10]; Jukka Kanerva, MD, PhD[11]; Marieke L. Kuijer, PhD[12]; Cristina Mendes, MD[13]; Evgenia Papakonstantinou, MD,

PhD[14]; Anna Raciborska, MD[15]; Vilma Rutkauskaite, MD[16]; Akmal Safwat, MD, PhD[17]; Sandra J. Strauss, MD PhD FRCP[18]; Gwen ML Sys, MD, PhD[19]; Claudia Valverde, MD[20]; Roelof van Ewijk, MD[21]; Esperanza Perez, MS[22]; Emanuela Palmerini, MD, PhD[23], (1) Gustave Roussy cancer campus, Paris, Ile-de-France, France, (2) SPAGN Sarcoma Patient Advocacy Global Network, Barcelona, Catalonia, Spain, (3) Anticancer Fund, Kingston upon Thames, England, United Kingdom, (4) Stockholm, Karolinska University Hospital, Stockholm, Stockholms Lan, Sweden, (5) Olgahospital Stuttgart, Stuttgart, Baden-Wurttemberg, Germany, (6) Oslo University Hospital, Oslo, Oslo, Norway, (7) Children's Health Ireland (CHI), Crumlin, Dublin, Ireland, (8) Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (9) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (10) St. Anna Children's Hospital and Children's Cancer Research Institute, Vienna, Wien, Austria, (11) HUS Helsinki University Hospital, New Children’s Hospital, Finland, Helsinki, Uusimaa, Finland, (12) Centre for Molecular Medicine Norway (NCMM), University of Oslo Leiden University Medical Center (LUMC), Leiden University, Oslo, Oslo, Norway, (13) Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Lisboa, Portugal, (14) Hellenic Society Of Pediatric Hematology- Oncology, Athens, Athen, Attiki, Greece, (15) Insitute of Mother and Child, Warsow, Mazowieckie, Poland, (16) Vilnius university hospitalSantaros KlinikosCenter for Pediatric Oncology and Hematology, Vilnius, Vilniaus Apskritis, Lithuania, (17) Danish centre for Particle Therapy, Aarhus University hospital , Aarhus, Midtjylland, Denmark, (18) UCL Cancer Institute, London, England, United Kingdom, (19) Ghent University Hospital, Ghent, Oost-Vlaanderen, Belgium, (20) Vall D’Hebron University Hospital, Barcelona, Catalonia, Spain, (21) Princess Maxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands, (22) Gustave Roussy, Villejuif, Ile-de-France, France, (23) IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy

Objective: Osteosarcoma, although a rare disease, is the most common primary bone cancer in adolescents and young adults, known to have a complex biology and an overall survival that has not improved over the last decades. Evolution towards metastatic, primarily pulmonary, disease remains the key problem despite heavy multi-modal therapy.

Our objective is to create a collaborative efforts for a better understanding of osteosarcoma biology, what's essential essential to tailor new therapies for the individual patient.

Methods: The FOSTER consortium (Fight Osteosarcoma Through European Research) was created to connects professionals of the full multidisciplinary team, including young investigators, and patient/parent advocates, at a European level.

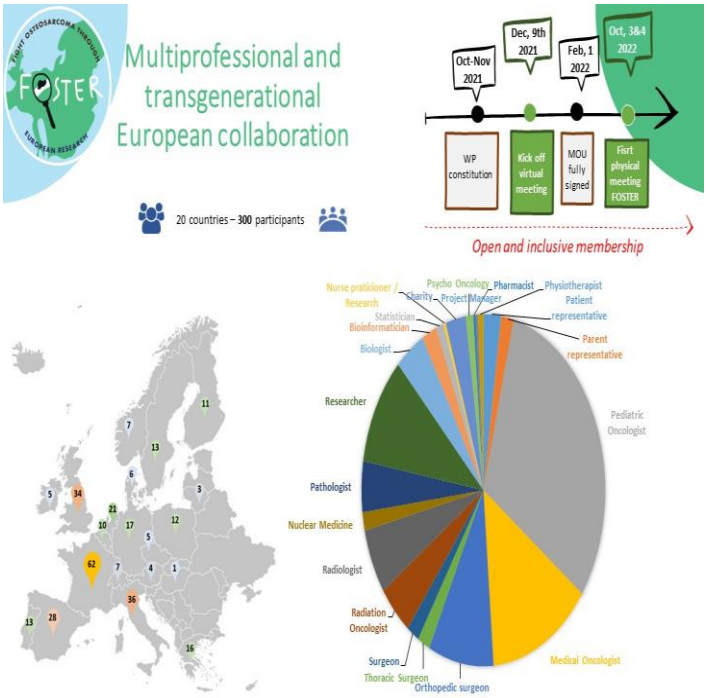
All members together aim to improve biological, translational and clinical research in osteosarcoma with the main objective to improve survival and quality of life of patients of all ages with osteosarcoma.

Results: The FOSTER consortium comprises 300 members from19 countries. It is organised in 8 work packages and 2 aditionnal groups, with an Executive Committee representing all countries, all professions, as well as patients and parents

The FOSTER consortium aims to integrate biological and preclinical studies in standard practice (WP1), to identify new promising therapies derived from molecular profiling programs and early phase clinical trial data (WP2), these to be implemented in optimally designed trials for relapsed disease (WP3) and where possible implemented in biologically stratified newly designed first line trials (WP4), with consideration of the preferred approach for local control of the primary tumor and metastatic lesions (WP5), improved radiological evaluation of the

disease and its response to therapy (WP6) and essential attention for quality of life, functional outcome and late effects (WP7). Data sharing for collaborative research and knowledge dissemination across professionals and patients/parents is key for the FOSTER consortium (WP8).

Conclusion: The FOSTER consortium is committed to improving outcomes for all osteosarcoma patients and has been structured explicitly to achieve this important goal, with strong patient and parent involvment. Without such a collaborative effort, the future for many osteosarcoma patients remains dismal.
The creation of a consortium in Europe in this rare disease is a first step for broader collaboration.



(P 74) SACRAL CHORDOMA: A RANDOMIZED & OBSERVATIONAL STUDY ON SURGERY VS DEFINITIVE RADIOTHERAPY IN PRIMARY LOCALIZED DISEASE (SACRO). INTERIM ANALYSIS

Stefano Radaelli, MD[1]; Maria Rosaria Fiore, MD[2]; Maria Grazia Petrongari, Prof, MD[3]; Alessandro Gasbarrini, Prof, MD[4]; Øyvind Sverre Bruland, Prof, MD[5]; Pilar Sancho, MD[6]; Jendrik Hardes, MD[7]; Susanne Scheipl, MD[8]; Beate Timmermann, MD[7]; Toru Akiyama, MD, PhD[9]; Vittorio Quagliuolo, MD[10]; Davide Maria M. Donati, MD[11]; Lorenzo Andreani, MD[12]; Josè Asencio, MD[13]; Ramona Verges, MD[14]; Viviana Appolloni, PhD[15]; Daniela Salvatore, Phd[16]; Gianluca Ignazzi, Master Degree[17]; Silvia Stacchiotti, MD[18]; Piero Fossati, MD[19]; Alessandro Gronchi, MD, FSSO[20], (1) Sarcoma Service, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (2) Fondazione CNAO -Centro Nazionale di Adroterapia Oncologica, Pavia, Italy, Pavia, Lombardia, Italy, (3) IRCCS Istituto Nazionale Tumori Regina

Elena, Rome, Italy, Rome, Lazio, Italy, (4) IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (5) Norwegian Radium Hospital, Oslo, Norway, Oslo, Oslo, Norway, (6) Hospital Universitario Virgen del Rocio, Sevilla, Spain, Sevilla, Andalucia, Spain, (7) University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (8) Univ. fur Orthopadie und Traumatologie Miedizinische Universitat Graz, Graz, Steiermark, Austria, (9) Saitama Medical Centre, Jichimedical University, Saitama, Saitama, Japan, (10) Humanitas, Research Hospital, Milan, Italy, Rozzano, Lombardia, Italy, (11) Istituto Ortopedico Rizzoli IOR IRCCS, SC Third Orthopaedic Clinic and Traumatology, Bologna Italy, Bologna, Emilia-Romagna, Italy, (12) Università di Pisa - Azienda Ospedaliera Universitaria Pisana, Pisa, Italy, Pisa, Toscana, Italy, (13) Hospital UniversitarioGregorio Maranon, Madrid, Spain, Madrid, Madrid, Spain, (14) Hospital Val D'Hebron, Barcelona, Spain, Barcelona, Catalonia, Spain, (15) Italian Sarcom Group, terni, Umbria, Italy, (16) Fondazione IRCCS Instituto Nazionale dei Tumori, Milano, Lombardia, Italy, (17) Italian Sarcoma Group, Torino, Piemonte, Italy, (18) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (19) MedAustron Ion Therapy Centre, Austria, Graz, Niederosterreich, Austria, (20) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy

Objective: Surgery with or without high-dose RT are the only curative treatments available for primary and localized sacral chordoma. Prospective studies comparing these two approaches have never been conducted and so far agreement on the optimal treatment strategy for these patients is lacking. The Italian Sarcoma Group (ISG) has promoted an international, prospective randomised/observational clinical trial aimed at identifying the best treatment modality for patients with primary localized sacral chordoma in terms of efficacy, side effects / quality of life (QoL) and long-term morbidities.

Methods: As from March 2017, patients are being enrolled at 16 centers worldwide. The following eligibility criteria are used: >/= 18 years, histologically confirmed diagnosis of chordoma (including positive immunostaining for nuclear brachyury), disease arising from the sacrum (from S1 to coccyx), primary and localized tumor amenable to receive either en-bloc resection with R0 margins surgery or definitive RT. Enrolled patients are asked to choose among 3 alternative options: surgery (+/- RT) (Arm A); definitive RT (Arm B), or randomization to either surgery (+/- RT) or definitive RT (Arm C). Definitive RT treatment can be delivered with three modalities: proton-therapy, mixed photon/proton RT, and carbon-ion RT. Study primary endpoint is relapse-free survival. Overall survival (OS) and QoL are secondary endpoints. This study is designed following a Bayesian statistical approach; the final objective of the statistical analysis will be to estimate a posterior probability distribution of the relative efficacy of the two treatment strategies being compared. The study is currently open to the accrual (ClinicalTrials.gov NCT02986516). The study is currently open to accrual; we report here the results of the second interim analysis (August 2022), while the 3rd interim analysis is ongoing and will be reported at the meeting.

Results: Between March 2017 and March 2023, 136 patients were enrolled in the trial: This exceedes the expected patient accrual, that was 122 (1.7 monthly). None of the patient accepted to be randomized and all patients chose to entered the observational cohorts (Arm A and B). Male patients are more represented in the study (N=94, 69%) and patient median age is 61 years. Median tumor size is 8.4 cm, with 76 (57%) and 60 (43%) tumors located above and below S3, respectively. Seventy-six (56%) and sixty (44%) patients underwent surgery (Arm A) and definitive RT (Arm B), respectively. Among patients treated with surgery, R0 margins were achieved in 81% of surgical resections, while 7% of patients had macroscopically incomplete resection. Among patients treated with definitive RT, 90% received carbon-ions 10% protons. The vast majority of patients (96%) received at least 73.6 Gy. Post-treatment morbidity was consistent with previously series for Arm A and B. At the cut-off date of the

second interim analysis (August 2022), 87 patients were evaluable. At a median follow up of 40 months (CI 95% 30-51 months), chordoma recurrences were observed in 4/38 (11%) and 10/49 (20.4%) patients in Arm A and B, respectively, with no statistically significant differences (log-rank test, P=0.757). Consequently, OS did not differ between patients enrolled in Arm A or B (Arm A: 2/38, 5.2%; Arm B: 3/49, 6.1%; log-rank test, P=0.408). The 3rd interim analysis is ongoing and will be reported at the meeting.

Conclusion: Actual accrual rate is above expectations. Interestingly, none of the 136 patients who have entered the study so far accepted to enter the randomized part of the study. According to the Bayesian study design interim analysis are provided yearly, without showing any statistically significant difference in recurrence and survival rate between wide en-bloc surgical resection or definitive RT. Therefore the study is continuing its recruitment and QoL date collection is ongoing.

(P 75) REAL-WORLD DATA ON REGORAFENIB AND CABOZANTINIB IN CHILDREN, ADOLESCENTS, AND YOUNG ADULTS WITH RELAPSED OSTEOSARCOMA AND EWING SARCOMA INCLUDED IN THE SACHA-FRANCE STUDY

Pablo Berlanga, MD/PhD[1]; Isabelle Aerts, MD PhD[2]; Nicolas André, MD/PhD[3]; Lee Aymar Ndounga-Diakou, PhD[4]; Chloe Puiseux, MD PhD[5]; Nadege Corradini, MD[6]; Stephane Ducassou, MD/PhD[7]; Marion Strullu, MD PhD[8]; Emilie De Carli, MD[9]; Natacha ENTZ-WERLE, MD, PhD[10]; Sandra Raimbault, MD[11]; Samuel Abbou, MD PhD[4]; Marianne Roumy, n/a[12]; Marjolaine Renouard, n/a[2]; Virginie Gandemer, MD PhD[13]; Salim Laghouati, PharmD[4]; Gilles vassal, MD, PhD[14], (1) Gustave Roussy cancer Centre, Villejuif, Ile-de-France, France, (2) Institut Curie, Paris, Ile-de-France, France, (3) La Timone Hospital, Marseilles, Provence-Alpes-Cote d'Azur, France, (4) Gustave Roussy Cancer Campus, Villejuif, Ile-de-France, France, (5) Gilles.Vassal@gustaveroussy.fr, Rennes, Bretagne, France, (6) Institut d'Hematologie et d'Oncologie Pédiatrique, Centre Léon Bérard, Lyon, Rhone-Alpes, France, (7) Pediatric Hospital, Beaurdeaux, Aquitaine, France, (8) Hopital Robert Debre, Paris, Ile-de-France, France, (9) Pediatric hospital of the University, Anger, Pays de la Loire, France, (10) University hospital of Strasbourg and UMR CNRS 7021, Strasbourg, Alsace, France, (11) Oscar Lambret Cancer Center, Lille, Nord-Pas-de-Calais, France, (12) University Hospital Angers, Angers, Pays de la Loire, France, (13) University Hospital Rennes, Rennes, Bretagne, France, (14) Gustave Roussy, Villejuif, Ile-de-France, France

Objective: Regorafenib and cabozantinib have shown anti-tumor activity in patients with bone sarcomas at disease recurrence. Nevertheless, activity and safety data are mainly reported in adult patients with limited information on children/adolescents and young adults. We here report the nationwide real-world anti-tumor activity and toxicity of regorafenib and cabozantinib in patients with relapsed osteosarcoma and Ewing sarcoma included in the SACHA-France study (NCT04477681).

Methods: The SACHA-France study is a prospective, multicenter, national observational registry, open in all French Society of Pediatric Oncology (SFCE) centers, that collects clinical safety and efficacy data on innovative anticancer therapies first approved in adults after 2007 that are administered to patients ≤ 25 years-old with pediatric malignancies (solid tumor or leukemia) and prescribed either as compassionate or off-label use. All regorafenib and cabozantinib prescriptions for patients with relapsed osteosarcoma and Ewing sarcoma from

01/01/2020 to 31/12/2022 included in the SACHA-France registry are here analyzed.

Results: In the study period, 516 anticancer therapies prescribed to 485 patients were collected in SACHA-France. Regorafenib and cabozantinib represented 10% of all prescriptions (n=52), mostly in patients with osteosarcoma and Ewing sarcoma (n=46). Forty-four prescriptions were made at the time of disease recurrence in 18 SFCE centers (median 2 patients per center, range: 1-6 patients). Median age at regorafenib/cabozantinib prescription was 16,8 years (range: 6,9-24,4), compared to 11.2 years (range: 0.25-24.9) in the SACHA-France population. An objective response (partial/complete response) was reported in 4 patients out 44 patients (9%). 4-months PFS was 45%, with a median PFS of 97 days (range: 14-521). Patient's characteristics and anti-tumor activity are presented in Table 1. Twelve patients (27%) continued with regorafenib/cabozantinib after tumor progression based on clinical benefit and/or lack of other therapies. In 23/44 patients (52%) at least a grade ≥2 clinical and/or grade ≥3 biological adverse drug reaction (ADR) was reported, leading to delayed therapy in 34% of patients. Overall, 5 serious ADRs were reported in 3 patients.

Conclusion: To our knowledge, this is the first comprehensive nation-wide study on regorafenib and cabozantinib prescriptions to children and adolescents and young adults with relapse osteosarcoma and Ewing sarcoma. In the real-life setting, safety profile is similar to that observed in other pediatric studies, with limited anti-tumor activity but prolonged clinical benefit in some patients.

Supported by Imagine for Margo, Association Hubert Gouin, Fondation du LEEM and the SFCE.

SACHA therapy	Tumor type (relapse)	N	Age at inclusion (years)	Prior lines of therapy	Time since diagnosis (years)	ORR	PFS (days)	Duration of therapy (days)	Patients still on therapy	Reason EOT
Cabozantinib	Osteosarcoma	11	16,2 (13,4-20,6)	2 (2-4)	1,7 (0,8-2,5)	1/10	88 (14-448) 4-m PFS: 27%	92 (14-448)	0	9 PD, 1 PD/AE, 1 AE not related
Regorafenib	Osteosarcoma	16	17,1 (6,9 - 22,8)	2 (-7)	1,6 (0,6-13,6)	0/16	135 (21-513) 4-m PFS: 56%	184 (21-513)	1	13 PD, 1 PD/AE, 1 SD (surgery)
Cabozantinib	Ewing	12	17,4 (9,3-24,4)	4 (1-9)	3,1 (1,5-7,1)	2/12	66 (18-210) 4-m PFS: 33%	129 (18-327)	2	9 PD, 1 PD/AE
Regorafenib	Ewing	5*	14,6 (10,5-17,8)	4 (2-6)	2,6 (1,3-5,1)	1/5	187 (98-388) 4-m PFS: 80%	189 (98-388)	1	3 PD, 1 PD/AE

Table 1: ORR: objective response rate, PFS: progression-free survival, EOT: end of treatment. Continuous variables are presented as median (range). * One patient received regorafenib combined with vincristine/irinotecan. Data cut off on 19/04/2023

(P 76) GENETIC EVIDENCE OF METACHRONOUS DEDIFFERENTIATION OF CHONDROSARCOMA AT A DISTANT SITE

Tetsuya Sekita, MD, PhD[1]; Akihiko Yoshida, MD, Ph.D[2]; Akira Kawai, MD, Ph.D[1]; Hitoshi Ichikawa, PhD[3]; Eisuke Kobayashi, MD[4], (1) National Cancer Center Hospital, Tokyo, Tokyo, Japan, (2) Department of Diagnostic Pathology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (3) National Cancer Center Research Institute, Tokyo, Tokyo, Japan, (4) Department of Musculoskeletal Oncology and Rehabilitation, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan

Objective: Dedifferentiated chondrosarcoma is a subtype of chondrosarcoma with a bimorphic histological appearance of a chondrosarcoma component with a transition to a high-grade, noncartilaginous sarcoma. It is particularly difficult to confirm the diagnosis when a sarcoma with no cartilaginous component occurs at a distant location from the primary lesion. We report a new emerging phenomenon of chondrosarcoma: metachronous dedifferentiation at a distant site with genetic evidence.

Methods: The patient was a 72-year-old woman with multiple lesions in the pelvis, lungs, and liver 18 months after resection of grade 2 central chondrosarcoma in the sternum. Imaging showed no cartilage component at any location. Although a needle biopsy from the pelvic region confirmed the diagnosis as high-grade sarcoma without a cartilage component, it was difficult to distinguish between a secondary sarcoma and metachronous metastatic lesions derived from dedifferentiated chondrosarcoma. Therfore, we compared the genomic alterations of the initial chondrosarcoma and the subsequent noncartilaginous sarcoma by whole exome sequencing (WES).

Results: Forty-three (30 nonsynonymous, 11 synonymous, one splicing junction, and one indel) and 57 (39 nonsynonymous, 13 synonymous, and 5 indel) somatic mutations were identified in the sternal resection specimen and the pelvic sarcoma specimen. Nineteen (14 nonsynonymous, 4 synonymous, and 1 indel) mutations were commonly found. In addition, the copy number analysis revealed highly similar aneuploidy between the two specimens, showing a wide range of chromosomal losses. Whole-chromosomal loss of heterozygosity (LOH) was commonly observed in 14 autosomes excluding Chr 2, 5, 7, 15, 16, 19, 20, and 21 in both samples. Multiple common somatic mutations and chromosomal losses indicated that sternal chondrosarcoma and pelvic noncartilaginous sarcoma were clonally related and that the pelvic tumor should represent a dedifferentiated chondrosarcoma arising from sternal grade 2 chondrosarcoma.

Conclusion: We evaluated the WES of central chondrosarcoma and metachronous dedifferentiated lesions. The possibility of metachronous dedifferentiation should be considered when a secondary sarcoma develops after central chondrosarcoma surgery, even if it arises in a different location from the primary tumor.

(P 77) PATIENT OUTCOMES AFTER OPEN VERSUS CORE NEEDLE BIOPSY IN LOWER EXTREMITY SARCOMA

Zachary D. Burke, MD[1]; Alexander Lazarides, MD[2]; Manit K. Gundavda, DNB(Orth)[3]; Anthony Griffin, MSc[4]; Kim Tsoi, MD, PhD[4]; Peter Ferguson, MSc., MD[5]; Jay S. Wunder, MD[4], (1) Celveland Clinic Sarcoma Program, Cleveland, OH, (2) Moffitt Cancer Center, Tampa, FL, (3) Kokilaben Hospital, Mumbai, Maharashtra, India, (4) Mount Sinai Hospital, Toronto, ON, Canada, (5) University of Toronto, Toronto, ON, Canada

Objective: There are limited data evaluating how the type of biopsy impacts definitive surgical resection or postoperative outcomes in bone sarcomas. The aims of this study were to (1) characterize current biopsy practice patterns, and (2) evaluate how biopsy type impacts outcomes.

Methods: This study was a preplanned secondary analysis of the prospective, multicenter PARITY (Prophylactic Antibiotic Regimens in Tumor Surgery) study. Patients with a benign diagnosis, metastatic disease, or no biopsy prior to surgery were excluded. Parametric and nonparametric tests were used to compare variables between groups, and the Cox proportional hazards method was used to compare infection-related and oncological outcomes at 1 year. Median functional outcome scores at 1 year were compared.

Results: In total, 464 patients met inclusion criteria from 48 sarcoma centers in 12 countries. CNB was used in 57.5% of cases. OB was more common in the U.S. and Canada. Median operative time was significantly longer after OB (324 versus 260 minutes; $p < 0.001$). In the OB group, significantly more skin ($p < 0.001$) and fascial tissue ($p < 0.001$) were excised with a lower rate of primary closure (86.3% versus 92.9%; $p = 0.03$). There were no differences in surgical site infection or oncological outcomes between the groups.

Conclusion: CNB was used most commonly overall. OB was more common in the U.S. and Canada. Patients undergoing OB had longer operative times, more excised tissue, and lower rates of primary closure. There were no differences in infection rates or oncological outcomes, including local recurrence.

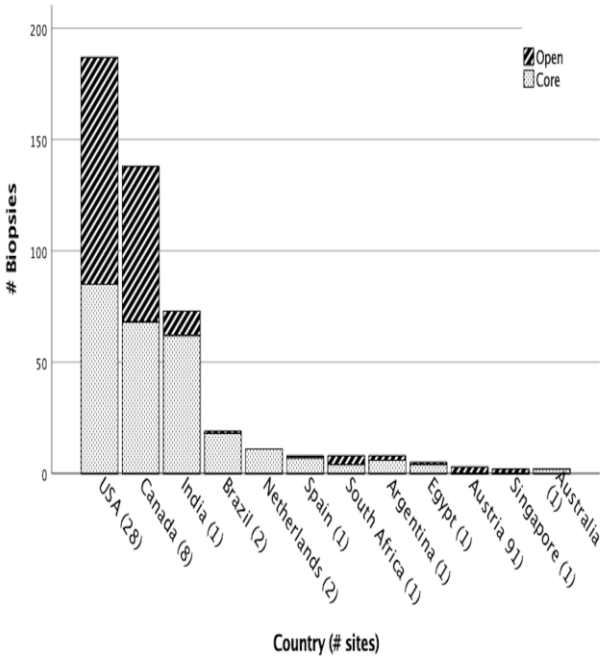


TABLE IV Oncological Outcomes at 1-Year Follow-up by Biopsy Modality

Oncological Variable	Total (N = 464) *(no. %)*	Open (N = 197) *(no. %)*	Core (N = 267) *(no. %)*	HR (95% CI)*	P Value*
Any oncological event	130 (28.0)	52 (26.4)	78 (29.2)	0.92 (0.64-1.31)	0.63
Local recurrence	31 (6.7)	14 (7.1)	17 (6.4)	1.18 (0.56-2.43)	0.65
Distant metastasis	110 (23.7)	42 (21.3)	68 (25.5)	0.85 (0.57-1.25)	0.40

*Cox proportional hazard method, with type of tumor and tumor histology included as covariates. HR = hazard ratio, and CI = confidence interval.

TABLE II Intraoperative Outcomes by Biopsy Modality*

Surgical Variable	Total (N = 464)	Open (N = 197)	Core (N =267)	P Value†
Operative time‡ *(min)*	271.0 (217.0-399.75)	324.0 (240.0-457.0)	260.0 (210.0-360.0)	**<0.001**
Skin excised§ *(no. [%])*				**<0.001**
None	144 (31.0)	29 (14.7)	115 (43.1)	
Small amount	230 (49.6)	107 (54.3)	123 (46.1)	
Moderate amount	58 (12.5)	41 (20.8)	17 (6.4)	
Large amount	29 (6.3)	19 (9.6)	10 (3.7)	
Muscle excised§ *(no. [%])*				0.99
None	33 (7.1)	14 (7.1)	19 (7.1)	
Small amount	207 (44.6)	87 (44.2)	120 (44.9)	
Moderate amount	134 (28.9)	58 (29.4)	76 (28.5)	
Large amount	85 (18.3)	35 (17.8)	50 (18.7)	
Fascia excised§ *(no. [%])*				**<0.001**
None	80 (17.2)	21 (10.7)	59 (22.1)	
Small amount	140 (30.2)	49 (24.9)	91 (34.1)	
Moderate amount	142 (30.6)	77 (39.1)	65 (24.3)	
Large amount	99 (21.3)	49 (24.9)	50 (18.7)	
Closure/reconstruction *(no. [%])*				
Primary closure	418 (90.1)	170 (86.3)	248 (92.9)	**0.03**
Local muscle flap and STSG	55 (11.9)	28 (14.2)	27 (10.1)	0.19
Local fasciocutaneous flap	8 (1.7)	6 (3.0)	2 (0.7)	0.08
Free flap	8 (1.7)	6 (3.0)	2 (0.7)	0.08

*Small amount is <5 cm^2, moderate amount is 5 to 10 cm^2, and large amount is >10 cm^2. †Chi-square test. Bold indicates a significant value ($p < 0.05$). ‡The values are given as the median, with the interquartile range (IQR) in parentheses. §Numbers shown are for patients with available data.

TABLE III Surgical Site Infection (SSI) at 1-Year Follow-up by Biopsy Modality

	Total (N = 464)	Open (N = 197)	Core (N = 267)	HR (95% CI)*	P Value*
Any SSI *(no. [%])*	86 (18.5)	30 (15.2)	56 (21.0)	0.72 (0.46-1.13)	0.15
Superficial incisional	21 (4.5)	10 (5.1)	11 (4.1)	1.18 (0.51-2.75)	0.70
Deep incisional	9 (1.9)	2 (1.0)	7 (2.6)	0.56 (0.23-2.97)	0.40
Organ/space	56 (12.1)	18 (9.1)	38 (14.2)	0.65 (0.37-1.14)	0.13

*Cox proportional hazard method, with type of tumor, preoperative chemotherapy, and tumor location included as covariates. HR = hazard ratio, and CI = confidence interval.

(P 78) THE CLINICAL OUTCOME OF EXTRASKELETAL MYXOID CHONDROSARCOMA

Chang-Bae Kong, MD, PhD[1]; Sang-Keun Woo, Ph.D.[2], (1) Korea Cancer Center Hospital, Seoul, Seoul-t'ukpyolsi, Republic of Korea, (2) Korea Institute of Radiological Medical Sciences, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Objective: Extraskeletal myxoid chondrosarcoma (EMC) is an extremely rare malignant mesenchymal neoplasm. accounting for less than 3% of soft tissue sarcoma. This sarcoma is usually characterized by an indolent course. The aim of our study was to document the clinical manifestation and oncologic outcomes of EMC.

Methods: This study identified 17 patients who were diagnosed and treated for EMC between January 2008 and December 2018. The identified cohort was then reviewed regarding age, gender, symptom onset, tumor location, MR images, surgical margin and pathologic diagnosis. The time to local recurrence and metastasis, follow-up duration, and the patients' final status were analyzed.

Results: The patients comprised 10 males and 7 female patients with a mean age of 54 (range, 31-79). The location of the tumor was buttock in 5, knee in 3, foot in 2, shoulder in 1, and back in 1. The average tumor diameter was 11.5 cm (range, 6-26 cm). At the time of diagnosis, 5 patients were AJCC stage II, 3 were IIIA, 3 were IIIB, and 6 were IV. Local recurrence occurred in 12 cases and distant metastasis occurred in 15 cases. The 5-year overall survival of patients with EMC was 73 ± 17%, and 2 patients died of the disease.

Conclusion: In spite of a high rate of local recurrence and distant metastasis, long-term survival rate in patients with EMC is quite high because of its indolent characteristics.

(P 79) HEAD AND NECK OSTEOSARCOMA: THE EXPERIENCE OF 52 CASES FROM A SINGLE TERTIARY INSTITUTION OVER 25 YEARS

Jae Sik Kim, MD, PhD[1]; Young In Kim, MD[2]; Ji Young Kim, MD[2]; Jin Ho Kim, MD, PhD[3], (1) Soonchunhyang University Seoul Hospital, Seoul, Seoul-t'ukpyolsi, Republic of Korea, (2) Seoul National University College of Medicine, Seoul, Seoul-t'ukpyolsi, Republic of Korea, (3) Seoul National University Hospital, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Objective: The present study aimed to estimate the survival outcomes of patients with head and neck osteosarcoma (HNOS) and investigate prognostic factors affecting treatment outcomes.

Methods: We reviewed 52 patients with pathologically confirmed HNOS (1996-2020), excluding radiotherapy (RT)-induced HNOS. All patients received curative surgery (S) with or without other treatment modalities (S alone, n=6, 11.5%; S+chemotherapy [CT], n=22, 42.3%; S+RT, n=3, 5.8%; and S+CT+RT, n=21, 40.4%). The median follow-up duration was 3.9 years.

Results: The mandible was the most common site of origin (n=32, 61.5%). Most patients had osteoblastic histology (n=28, 53.8%). The 2-year overall (OS) and disease-free survival (DFS) rates were 79.3% and 55.5%, respectively. Eighteen (34.6%) and three (5.8%) patients

experienced local and regional recurrence with a 2-year locoregional relapse-free survival of 68.7%. Distant metastasis was identified in 17 patients (32.7%) with a 2-year distant metastasis-free survival of 67.5%. Lung (n=11, 21.2%) was the most commonly involved. On univariate analysis, age >40 years (p=0.034) and RT (p=0.037) were risk factors for poorer OS. Tumors >3.7 cm were associated with poorer DFS (p=0.041). Among 17 patients receiving adjuvant RT, unplanned prolongation of RT ≥5 days was associated with poorer OS (p=0.003) and DFS (p=0.013).

Conclusion: The benefit of combined modalities was not clear for surgically treated HNOS patients in the current study. The risk of local recurrence was comparable to that of distant metastasis, and the role of adjuvant RT warrants further investigation. It would be safe to limit any unplanned RT prolongation to less than 5 days.

(P 80) OPTIMIZING DOSE OF MAP THERAPY FOR CHILDREN, YOUNG ADULTS AND ADULTS WITH OSTEOSARCOMA; A SINGLE-CENTRE RETROSPECTIVE ANALYSIS

Bunpei Miyazaki, MD[1]; Yuki Kojima, MD, Ph.D[2]; Ayumu Arakawa, MD[3]; Shintaro Iwata, MD[4]; Eisuke Kobayashi, MD[5]; Kazuki Sudo, MD, Ph.D[2]; Tatsunori Shimoi, MD, Ph.D[2]; Akihiko Yoshida, MD, Ph.D[6]; Chitose Ogawa, MD[3]; Akira Kawai, MD, Ph.D[4]; Kan Yonemori, MD, Ph.D[2], (1) Department of Pediatric Oncology and Medical Oncology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (2) Department of Medical Oncology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (3) Department of Pediatric Oncology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (4) National Cancer Center Hospital, Tokyo, Tokyo, Japan, (5) Department of Musculoskeletal Oncology and Rehabilitation, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (6) Department of Diagnostic Pathology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan

Objective: MAP (Methotrexate: MTX, Adriamycin: ADR, Cisplatin: CDDP) therapy is a standard chemotherapy for patients with osteosarcoma across all ages. Although adult patients are more toxic to anticancer therapy than pediatric patients, and thus dose intensities cannot be maintained, the dose intensity that is appropriate for each age group is not clear. We performed a retrospective analysis of patients with osteosarcoma to investigate whether the dose reduction of MAP therapy affects the efficacy and feasibility in patients with osteosarcoma in each age group.

Methods: We performed a single-center retrospective chart review analysis. Patients who were histologically diagnosed with osteosarcoma and received MAP therapy from January 1, 2014 to December 31, 2021 were included. Patients with recurrence, those who previously received MAP therapy at other hospitals, and those who received other agents such as ifosfamide or radiotherapy were excluded. We classified the patients as pediatric (PED, 0-19 years old), young adults (YA, 20-39 years old), and older adults (OA, 40 years old and older). Patients were classified according to the doses per cycle (DPC) they were scheduled to receive and the dose intensity (DI) they received for each agent. To calculate DI, we used DI-200, which is the total amount of the agent a patient received by day 200 of chemotherapy. We evaluated the relapse-free survival (RFS), therapy-complete rate (TCR), and serious adverse events in each age group, DPC, and DI-200. For the DI-200 analysis, only patients who survived to day 200 of

chemotherapy without disease progression or relapse were included. This study was approved by our institutional ethical review board.

Results: During the study period, 78 osteosarcoma patients received MAP therapy in our hospital. Of these, 24 patients were excluded (7 relapsed, 4 previously received MAP therapy in other hospitals, 13 received other agents), and the remaining 54 patients were analyzed. There were 24 PED patients, 19 YA patients, and 11 OA patients. Significant differences were observed in the proportion of patients with a primary site other than extremity (PED 9 %, YA 52 %, OA 81 %, p< 0.01), the median number of preoperative chemotherapy cycles (PED 6, YA 5, OA 0, p< 0.01), and metastases at diagnosis (PED 29 %, YA 15 %, OA 18 %, p< 0.01). The median DPC of MTX was 12 g/m2 in PED, 10 g/m2 in YA, and 8 g/m2 in OA patients, respectively. The median DPC of CDDP was 120 mg/m2 in PED and YA, and 80 mg/m2 in OA patients, respectively. All patients except the one patient received ADR 60 mg/m2 per cycle. The median DI-200 was 80 g/m2 for MTX, 330 mg/m2 for ADR, and 480 mg/m2 for CDDP, respectively. The median MTX DI-200 was 96 g/m2 in PED, 72 g/m2 in YA, and 42.3 g/m2 in OA (p< 0.01). The median CDDP DI-200 was 480 mg/m2 in PED, 460 mg/m2 in YA, and 370 mg/m2 in OA (p< 0.01). There was no statistical significance in RFS across age groups, DPC and DI-200 of each agent. We compared RFS stratified by DPC and DI-200 in each age group, and the higher dose group in DPC and DI-200 did not show superiority. On multivariate analysis of RFS, neither the age group, DPC and DI-200 showed statistical significance. For TCR, the OA patient group was statistically lower. Renal dysfunction and delayed elimination of MTX were identified as the main reasons for the discontinuation of MAP therapy. There was no statistical significance for serious adverse events when compared by age group and DPC.

Conclusion: The optimal dose of MAP therapy may differ among age groups. A prospective study in all age groups is needed to determine the optimal dose of MAP therapy.

(P 81) OPTIMIZING EWING SARCOMA AND OSTEOSARCOMA BIOPSY ACQUISITION: CONSENSUS RECOMMENDATIONS FROM THE CHILDREN'S ONCOLOGY GROUP BONE TUMOR COMMITTEE

Matthew S. Dietz, DO, MSEd[1]; Susan Whiteway, MD[2]; Ajay Gupta, MD, MS[3]; Jessica L. Davis, MD[4]; Nicole Montgomery, MD[5]; Sarah Cohen-Gogo, MD, PhD[6]; Christine M. Heske, MD[7]; Emily K. Slotkin, MD[8]; C. Matthew Hawkins, MD[9]; Brian T. Craig, MD[10]; Alyaa Al-Ibraheemi, MD[11]; Roshni R. Dasgupta, MD MPH[12]; David S. Geller, MD[13]; David S. Shulman, MD[14]; Safia K. Ahmed, MD[15]; Danny J. Indelicato, MD[16]; Catherine M. Albert, MD[17]; Jesse K. Sandberg, MD[18]; Elyssa Rubin, MD[19]; Elizabeth R. Lawlor, MD, PhD[20]; Leo Mascarenhas, MD, MS[21]; Patrick J. Grohar, MD, PhD[22]; Odion Binitie, MD[23]; Damon Reed, MD[24]; Katherine A. Janeway, MD[14]; Ryan D. Roberts, MD, PhD[25]; Steven G. Dubois, MD, MS[26]; Michael Isakoff, MD[27]; Kelly M. Bailey, MD, PhD[28], (1) University of Utah, Primary Children's Hospital, Salt Lake City, UT, (2) Walter Reed National Military Medical Center, Bethesda, MD, (3) Roswell Park Oishei Children's Cancer and Blood Disorders Program, Buffalo, NY, (4) Indiana University, Indianapolis, IN, (5) Texas Childrens Hospital, Houston, TX, (6) The Hospital for Sick Children, Toronto, ON, Canada, (7) National Cancer Institute, Bethesda, MD, (8) Memorial Sloan Kettering Cancer Center, New York, NY, (9) Children's Healthcare of Atlanta - Emory University School of Medicine, Atlanta, GA, (10) Children's Wisconsin, Medical College of

Wisconsin, Milwaukee, WI, (11) Boston Children's Hospital, Boston, MA, (12) Cincinnati Children's Hospital Medical Center, Cincinnati, OH, (13) Montefiore Medical Center - Orthopaedic Oncology, Bronx, NY, (14) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA, (15) Mayo Clinic Arizona, Phoenix, AZ, (16) UFPTI, Jacksonville, FL, (17) Seattle Children's Hospital, University of Washington, Fred Hutchinson Cancer Center,, Seattle, WA, (18) Lucile Packard Children's Hospital, Stanford University, Stanford, CA, (19) Children's Hospital of Orange County, Orange, CA, (20) Seattle Children's Research Institute, Seattle, WA, (21) Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, (22) Children's Hospital of Philadelphia, Philadelphia, PA, (23) H. Lee Moffitt Cancer Center, Tampa, FL, (24) Moffitt Cancer Center, Tampa, FL, (25) Nationwide Children's Hospital, Columbus, OH, (26) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA, (27) Connecticut Children's Medical Center, Hartford, CT, (28) University of Pittsburgh School of Medicine, Pittsburgh, PA

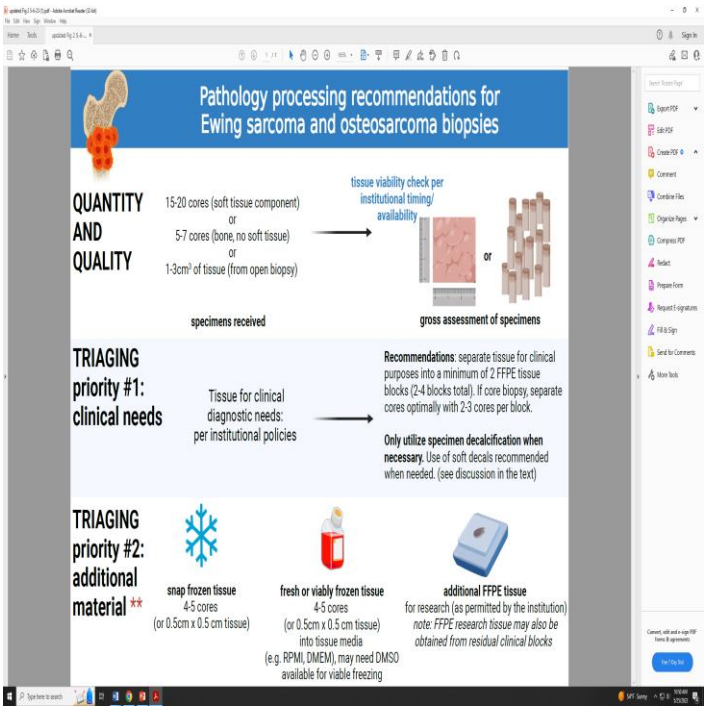
Objective: Osteosarcoma and Ewing sarcoma are rare, aggressive bone and soft tissue sarcomas with peak incidence in adolescents and young adults. Novel risk-stratified treatment approaches for bone sarcoma are emerging and molecular profiling will be an essential component to inform future clinical trial enrollment and disease management. The procurement of sufficient tumor samples to facilitate downstream diagnostic, clinical molecular, and research-based testing are key to these efforts.
Percutaneous image-guided biopsy techniques have been increasingly employed for the diagnosis of primary bone tumors and confirmation of disease progression. Percutaneous procedures are less invasive and reduce recovery times, however potential limitations exist as the quantity and quality of tissue obtained through these procedures can vary. Further, sample processing requirements of bone tumors relative to other tumor types heightens the need for a standardized approach to tissue collection. As the bone tumor field moves toward an era of risk-stratified and molecular-based treatment options for patients, ensuring the acquisition of adequate tumor material is a priority. In this context, the Children's Oncology Group (COG) Bone Tumor Committee details expert-vetted guidance for the acquisition and subsequent processing and storage of biopsy specimens for Ewing sarcoma and osteosarcoma.

Methods: The COG Bone Tumor Committee convened a multidisciplinary group of experts to evaluate available literature and institutional practices to generate guidance for the acquisition of suspected Ewing sarcoma and osteosarcoma biopsy material. The group included representation from oncology, pathology, orthopedic oncology, interventional radiology, basic science researchers and patient advocates. Members included representation from small, medium and large North American oncology programs to ensure. feasibility of universally implementing recommendations. Current and near-future biopsy tissue requirements were reviewed and are reflected in the consensus recommendations for pre-biopsy planning, percutaneous biopsy approaches and open biopsy approaches as well as biopsy tissue processing, allocation and storage.

Results: When a diagnosis or relapse of osteosarcoma or Ewing sarcoma is suspected, recommendations include pre-biopsy planning with a multidisciplinary team to ensure requisite tissue specimens are acquired and patient safety is prioritized. The biopsy location must be identified by the treating surgeon and planned in line with future incisions.
For core biopsies, we recommend the following: 15-20 16G 1.5-3cm cores for tumors with a soft tissue component; 5-7 12-13G cores for tumors without a soft tissue component and, when osteosarcoma is suspected, acquire an additional 2-3 12-13G cores of the underlying osteoid. This yield is anticipated to address all clinical and research/clinical trial-based needs including 2-4 blocks with 2-3 cores per block for clinical diagnostic needs, snap frozen tissue (~0.5 grams per vial), and fresh or viably frozen tumor material (Figure 1).

For open biopsies, a minimum of 1 cubic centimeter should be obtained. It is critical to adhere to principles for performing safe open biopsies including: use of a small longitudinal incision, maintenance of hemostasis, minimizing dissection, limited drain use and avoidance of neurovascular structures.
Pathology handling of bone biopsies is key. We suggest decalcifying tissue only if necessary. When necessary, we recommend the use of “soft decals” such as ETDA or ETDA/formic acid combinations for better preservation of nucleic acid integrity. For routine diagnostic processing, clinical tumor tissue specimens are fixed in formalin and embedded in paraffin (FFPE). While much improvement has occurred in the ability to extract nucleic acids from FFPE, there are some limitations to testing post-fixation. Therefore, it is beneficial to preplan the allocation of tissue for snap freezing, fresh tissue, and viable preservation of specimens with 10% DMSO-containing media.

Conclusion: This guidance reflects general approaches for consideration based on the perspective of clinical and scientific experts in the field in North America, with the understanding that individual clinical decisions regarding tissue acquisition must be made based on tumor location and patient condition. Identification of requisite tumor biopsy volumes and harmonization of pathology processing is paramount to prospective research validation efforts to advance both bone sarcoma clinical care and research. Further, novel therapeutic agents are routinely introduced with companion biomarkers, thus it is expected that molecular biomarker testing will become the standard of care. This perspective aims to serve as a reference to facilitate multidisciplinary harmonization in biopsy tissue acquisition (initial diagnosis and relapse specimens) and processing algorithms across institutions. Optimizing tissue collection to power innovative clinical trials informed by disease biology and to better understand treatment resistance mechanisms in advanced disease is paramount toward improving the care of children, adolescents and young adults diagnosed with Ewing sarcoma and osteosarcoma.



(P 82) PROGNOSTIC FACTORS AND OUTCOMES OF 275 PATIENTS WITH HEAD AND NECK BONE AND SOFT TISSUE SARCOMA: A SINGLE CENTRE COHORT STUDY

Mark See[1]; Chen Ee Low[2]; Chun En Yau[2]; Ru Xin Wong, MBBS, FRCR (Clinical Oncology)[3]; Whee Sze Ong, MAppStats[4]; Valerie S. Yang, MB BChir (Cantab), PhD (Cantab), MRCP (UK), FAMS (Medical Oncology) (Clinician Scientists)[3], (1) Lee Kong Chian School of Medicine, Nanyang Technological Institution, Singapore, N/A, Singapore, (2) Yong Loo Lin School of Medicine, National University of Singapore, SIngapore, N/A, Singapore, (3) Division of Medical Oncology, National Cancer Centre Singapore, Singapore, N/A, Singapore, (4) Division of Clinical Trials and Epidemiological Sciences, National Cancer Centre Singapore, Singapore, N/A, Singapore

Objective: Head and neck sarcomas (HNS) are a heterogenous group of cancers that are surgically and medically difficult to treat. Despite their low incidence (1% of head & neck malignancies, 5% of all sarcomas), outcomes worldwide are dismal with poor overall survival and high local recurrence rates, prompting urgent attention. Due to the rarity and diversity of HNS, our current understanding of HNS prognosis and treatment is limited. Moreover, the existing body of knowledge mostly comprises published studies describing HNS within predominantly Western populations, and may not adequately represent other cohorts around the world. This study aims to expand the collective understanding of head and neck sarcomas (HNS) and their prognostic factors within a Southeast Asian context.

Methods: 275 HNS patients seen at the National Cancer Centre Singapore were identified from the institution's database. Overall survival (OS) and progression free Interval (PFI) were calculated using the Kaplan-Meier method. OS was defined as time from the date of diagnosis till the date of patient death. PFI was defined as time from the date of diagnosis till the date of disease recurrence or progression. Recurrence was identified in patients who presented with localised disease at diagnosis without gross residual tumour post-resection, or had complete response to radiotherapy and/or chemotherapy. Progression was identified in patients who did not meet these criteria. Between group survival comparisons were conducted using the log-rank test. Prognostic factors were identified via Cox proportional hazards regression analyses.

Results: Median follow-up time was 39 months (95%CI 25.5, 50.9). Overall mean age was 55.2 years (standard deviation, 20.4 years). Majority of the patients (73.5%) were of Chinese ethnicity. Indians and Malays each made up 5.8% of the cohort, while the remaining 14.9% consisted of other minority races. The 3-year OS and PFI was 52.7% (95%CI 46.0%, 60.3%) and 47.1% (95%CI 39.6%, 56.0%) respectively. Multivariate Cox regression analysis revealed increasing age (HR 1.04, 95%CI 1.02, 1.05), absence of distant metastasis at diagnosis (HR 0.24, 95%CI 0.14, 0.39), radiotherapy induced disease (HR 3.02, 95%CI 1.71, 5.31), and wide surgical excision (HR 0.42, 95%CI 0.24, 0.73) as independent prognostic factors for OS. Primary disease sited at the scalp (HR 2.37, 95%CI 1.07, 5.28; relative to bone) and absence of distant metastasis at diagnosis (HR 0.10, 95%CI 0.05, 0.18) were identified as independent prognostic factors for PFI.

Conclusion: Our results support wide surgical excision in treating HNS within our cohort. We have also identified other factors of significant prognostic value. Compared with bone sarcomas, only soft tissue sarcoma with primary disease sited at the scalp demonstrated a significant difference in patient outcomes in our analysis. Interestingly, the use of chemotherapy and radiotherapy did not result in any significant difference in patient outcomes. Overall, this study represents one of the largest single-institution cohorts in the literature to date, and provides crucial insights towards predicting HNS outcomes. Long term follow up and further

analysis are required to integrate our identified prognostic factors into risk stratification algorithms and optimise treatment.

Table 1: Cohort characteristics (n = 275)

Characteristics	n	%
Patient Factors		
Age, years	55.2 (20.4)*	
Gender		
Male	153	55.6
Female	122	44.4
Race		
Chinese	202	73.5
Indian	16	5.8
Malay	16	5.8
Others	41	14.9
Disease Factors		
Primary disease site		
Bone	44	16.0
Face, parotids, jaw, and neck	63	22.9
Scalp	80	29.1
Skin	31	11.3
Upper aerodigestive tract	57	20.7
Distant metastasis at diagnosis		
Present	41	14.9
Absent	234	85.1
Location of metastasis at diagnosis		
None	234	85.1
Other sites	21	7.6
Lymph nodes	20	7.3
Disease caused by Radiotherapy		
No	244	88.7
Yes	31	11.3
Treatment Factors		
Wide surgical excision		
No	112	40.7
Yes	163	59.3
Chemotherapy		
Yes	90	32.7
No	185	67.3
Chemotherapy intent		
Adjuvant	26	9.4
Palliative	64	22.3
Not done	185	67.3
Chemotherapy Regime		
Combination	45	16.4
Single Agent	43	15.6
Not done	185	67.3
Unknown	2	0.7
Radiotherapy		
Yes	109	39.6
No	166	60.4
Radiotherapy Intent		
Adjuvant	48	17.5
Palliative	61	22.2
Not done	166	60.8

*Data presented is mean (standard deviation)

Table 2: Prognostic factors for Overall Survival

Variable	Univariate analyses			Multivariable analyses		
	HR	95% CI	p-value	HR	95% CI	p-value
Patient factors						
Age, per year increase	1.04	1.03, 1.05	**<0.001**	1.03	1.02, 1.05	**<0.001**
Gender						
Female	1					
Male	1.08	0.76,1.54	0.668			
Race						
Chinese	1					
Indian	0.54	0.24, 1.23	0.14			
Malay	0.68	0.23, 1.54	0.35			
Others	0.39	0.18, 0.84	**0.02**			
Disease Factors						
Primary disease site						
Bone	1			1		
Face, parotids, jaw, and neck	1.18	0.61, 2.28	0.6170	0.82	0.40, 1.68	0.593
Scalp	2.87	1.59, 5.17	**<0.001**	1.27	0.59, 2.73	0.543
Skin	0.73	0.31, 1.68	0.4553	0.49	0.19, 1.26	0.138
Upper aerodigestive tract	1.55	0.80, 3.02	0.1898	1.06	0.51, 2.21	0.880
Distant metastasis at diagnosis						
Present	1			1		
Absent	0.20	0.13, 0.30	**<0.001**	0.24	0.14, 0.39	**<0.001**
Location of metastasis at diagnosis						
None	1					
Other sites	9.11	5.17, 16.0	**<0.005**			
Lymph nodes	3.35	1.84, 6.10	**<0.005**			
Radiotherapy induced disease						
No	1			1		
Yes	1.89	1.17, 3.05	**0.0098**	3.02	1.71, 5.31	**<0.001**
Treatment Factors						
Wide surgical excision						
No	1			1		
Yes	0.24	0.17, 0.35	**<0.001**	0.42	0.24, 0.73	**0.002**
Chemotherapy intent						
Not done	1			1		
Adjuvant	0.65	0.32, 1.30	0.222	1.31	0.60, 2.88	0.499
Palliative	2.14	1.46, 3.13	**0.001**	0.78	0.48, 1.25	0.304
Chemotherapy Regime						
Combination	1					
Single Agent	1.95	1.13, 3.42	**0.0164**			
Not done	0.90	0.56, 1.49	0.6603			
Unknown	0.71	0.01, 5.13	0.7992			
Radiotherapy Intent						
Note done	1			1		
Adjuvant	0.62	0.37, 1.04	0.070	0.80	0.45, 1.41	0.436
Palliative	2.73	1.85, 4.01	**0.001**	1.04	0.66, 1.64	0.875

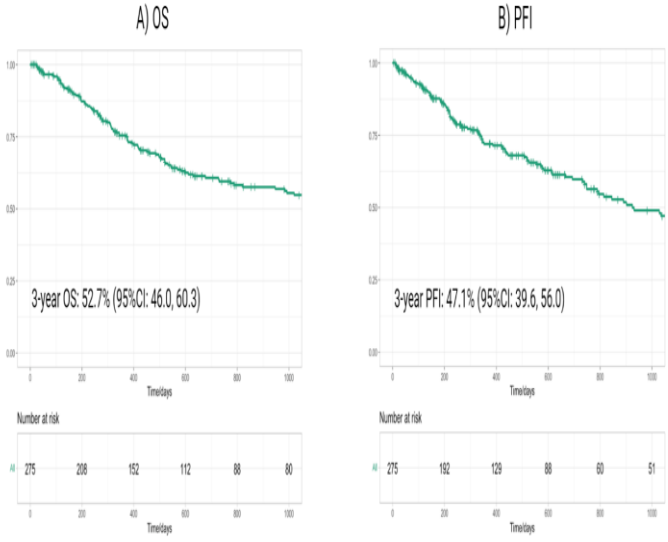
Bolded values indicate p <0.05
HR: Hazard Ratio
CI: Confidence Interval

Table 3: Prognostic factors for Progression Free Interval

Variable	Univariate analyses			Multivariable analyses		
	HR	95% CI	p-value	HR	95% CI	p-value
Patient factors						
Age, per year increase	1.01	1.00, 1.02	**0.009**	1.01	1.00, 1.02	0.170
Gender						
Female	1					
Male	1.29	0.88, 1.91	0.194			
Race						
Chinese	1					
Indian	0.56	0.23, 1.40	0.218			
Malay	0.90	0.42, 1.95	0.793			
Others	1.07	0.62, 1.87	0.799			
Disease Factors						
Primary disease site						
Bone	1			1		
Face, parotids, jaw, and neck	1.42	0.71, 2.82	0.322	1.47	0.69, 3.11	0.320
Scalp	2.54	1.32, 4.89	**0.005**	2.37	1.07, 5.28	**0.034**
Skin	0.87	0.37, 2.08	0.7607	0.93	0.36, 2.41	0.888
Upper aerodigestive tract	2.03	1.02, 4.04	**0.0447**	1.46	0.68, 3.15	0.334
Distant metastasis at diagnosis						
Present	1			1		
Absent	0.09	0.06, 0.15	**<0.001**	0.10	0.05, 0.18	**<0.001**
Location of metastasis at diagnosis						
None	1					
Other sites	14.98	8.32, 26.9	**<0.005**			
Lymph nodes	8.21	4.46, 15.1	**<0.005**			
Radiotherapy induced disease						
No	1			1		
Yes	0.86	0.45, 1.65	0.644	1.09	0.54, 2.19	0.580
Treatment Factors						
Wide surgical excision						
No	1			1		
Yes	0.44	0.29, 0.65	<0.001	1.19	0.65, 2.18	0.580
Chemotherapy intent						
Not done	1			1		
Adjuvant	1.20	0.64, 2.23	0.570	1.88	0.92, 3.81	0.082
Palliative	2.84	1.86, 4.35	**<0.001**	1.12	0.64, 1.99	0.689
Chemotherapy Regime						
Combination	1					
Single Agent	1.56	0.88, 2.73	0.1249			
Not done	0.58	0.36, 0.93	**0.0249**			
Unknown	1.33	0.18, 9.87	0.7801			
Radiotherapy Intent						
Note done	1			1		
Adjuvant	0.88	0.53, 1.46	0.628	0.88	0.51, 1.52	0.652
Palliative	2.65	1.71, 4.11	**<0.001**	1.28	0.74, 2.21	0.379

Bolded values indicate p <0.05
HR: Hazard Ratio
CI: Confidence Interval

Figure 1: Kaplan-Meier Curves for Overall Survival (A) and Progression Free Interval (B)



(P 83) CLINICO-GENOMIC PROFILING OF CONVENTIONAL AND DEDIFFERENTIATED CHONDROSARCOMAS REVEALS TP53 MUTATION TO BE ASSOCIATED WITH WORSE OUTCOMES

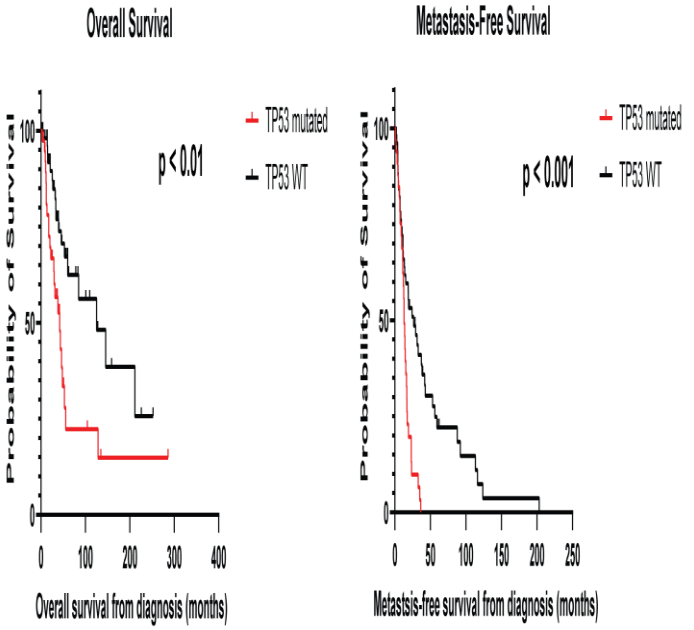
Ryan A. Denu, MD, PhD[1]; Richard K. Yang, MD, PhD[1]; Alexander J. Lazar, MD, PhD[2]; Shalin Shreyaskumar Patel, MD[2]; Valerae Lewis, MD[2]; Jason Roszik, PhD, MBA[1]; Andy Livingston, MD, MS[2]; Wei-Lien Wang, M.D[2]; Kenna R. Shaw, PhD[1]; Ravin Ratan, MD[2]; Maria A. Zarzour, MD[1]; Justin Bird, MD[2]; Shaan M. Raza, MD[1]; Kadir C. Akdemir, PhD[1]; Jordi Rodon Ahnert, MD, PhD[1]; Vivek Subbiah, MD[1]; Shreyaskumar Patel, MD[2]; Anthony P. Conley, MD[1], (1) MD Anderson Cancer Center, Houston, TX, (2) The University of Texas MD Anderson Cancer Center, Houston, TX

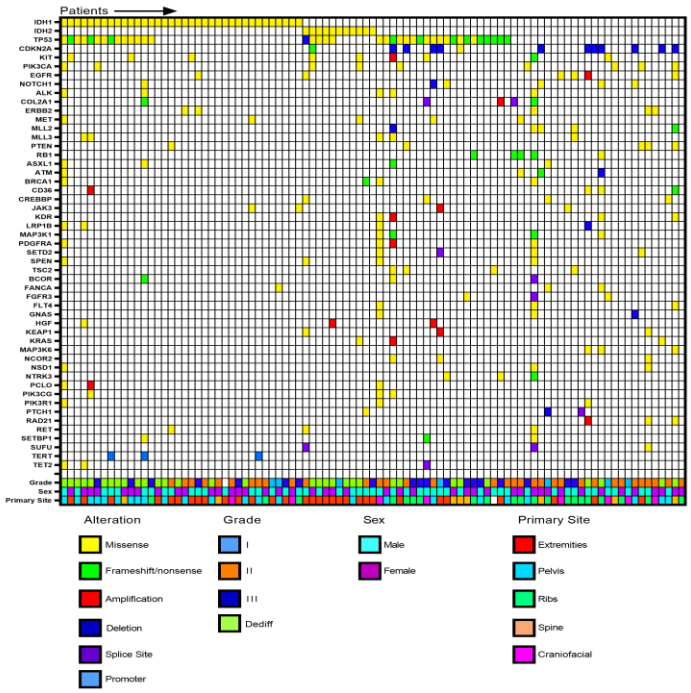
Objective: Chondrosarcomas are the most common primary bone tumor in adults. IDH1 and IDH2 mutations are prevalent. We aimed to assess the clinico-genomic properties of IDH mutant versus IDH wild type (WT) chondrosarcomas as well as alterations in other genes.

Methods: We included 93 patients with conventional and dedifferentiated chondrosarcoma for which there was available clinical next generation sequencing data. Clinical and genomic data were extracted and compared between IDH mutant and IDH WT chondrosarcomas and between TP53 mutant and TP53 WT chondrosarcomas.

Results: As expected, tumor grade was a strong predictor of survival. IDH1 and IDH2 mutations are prevalent in chondrosarcoma (50.5%), more common in chondrosarcomas arising in the extremities, associated with higher age at diagnosis, and more common in dedifferentiated chondrosarcomas compared to grades 1-3 conventional chondrosarcoma. There was no difference in survival based on IDH mutation in univariate and multivariate analyses. TP53 mutation was the next most prevalent (41.9%) and is associated with worse overall survival and metastasis-free survival in both univariate and multivariate analyses (figure). A personal history of other cancers was seen in 17.2% of chondrosarcoma patients.

Conclusion: IDH mutations are prevalent in chondrosarcoma though were not associated with survival outcomes in this cohort. TP53 mutations were the next most common alteration and were associated with worse outcomes.


Overall Survival
Probability of Survival
100
50
0
p < 0.01
TP53 mutated
TP53 WT
0
100
200
300
400
Overall survival from diagnosis (months)
Metastasis-Free Survival
Probability of Survival
100
50
0
p < 0.001
TP53 mutated
TP53 WT
0
50
100
150
200
250
Metastsis-free survival from diagnosis (months)


Patients
IDH1
IDH2
TP53
CDKN2A
KIT
PIK3CA
EGFR
NOTCH1
ALK
COL2A1
ERBB2
MET
MLL2
MLL3
PTEN
RB1
ASXL1
ATM
BRCA1
CD36
CREBBP
JAK3
KDR
LRP1B
MAP3K1
PDGFRA
SETD2
SPEN
TSC2
BCOR
FANCA
FGFR3
FLT4
GNAS
HGF
KEAP1
KRAS
MAP3K6
NCOR2
NSD1
NTRK3
PCLO
PIK3CG
PIK3R1
PTCH1
RAD21
RET
SETBP1
SUFU
TERT
TET2
Grade
Sex
Primary Site
Alteration
Missense
Frameshift/nonsense
Amplification
Deletion
Splice Site
Promoter
Grade
I
II
III
Dediff
Sex
Male
Female
Primary Site
Extremities
Pelvis
Ribs
Spine
Craniofacial

(P 84) CSF1R OVEREXPRESSION ACCELLERATES OSTEOSARCOMA TUMOR FORMATION AND GENERATES TUMORS WITH DECREASED IMMUNE CELL INFILTRATION

Aaron L. Sarver, PhD[1]; Justin Tibbitts, MS[2]; Nuri A. Temiz, PhD[1]; Wendy Hudson, Ph.D.[1]; David A. Largaespada, PhD[1], (1) University of Minnesota, Minneapolis, MN, (2) University of Minnesota, minneapolis, MN

Objective: Understanding the molecular events responsible for transcriptional variation (heterogeneity) within osteosarcoma tumors is challenging, especially considering the extreme inter-tumor genomic and transcriptional heterogeneity in this rare and deadly cancer. The objective of this study was to investigate the underlying mechanisms of transcriptional variation in osteosarcoma using genetically engineered mouse models.

Methods: To address this objective in an unbiased manner, we employed a genetically engineered mouse model where an oncogenic transposon was mobilized using the Osx1-Cre transgene. This model allowed for the accelerated formation of osteosarcoma tumors in both wild-type and Trp53R270H background mice, regardless of their sex. RNA-Seq data was generated from the resulting tumors, including those formed without transposon mobilization in Trp53R270H mutant mice. By analyzing the RNA-Seq data, we identified transposon-gene fusions that acted as drivers of tumor formation. Systematic analyses of transcript levels using GCESS dimensional reduction revealed conserved transcriptional variation in the genetically engineered mouse tumors, as well as in endogenously derived human and canine osteosarcoma tumors. By comparing the observed transcriptional patterns with the recurrent fusions, we generated hypotheses regarding the role of the driver events in tumor formation, which were further validated via generation of additional genetically engineered mice.

Results: Recurrent fusions were observed in the transposon-accelerated mouse tumors, and these fusions varied based on genetic background, sex, and original chromosome location of the transposon. However, no strong differences were observed in the tumor transcriptional patterns based on the Sleeping Beauty (SB) acceleration, genetic background, sex, or original chromosome location of the transposon. Comparison of the fusions with the observed transcriptional patterns suggested that overexpression of Csf1r was oncogenic and immunosuppressive, due to a decrease in the presence of immune-related transcripts. Csf1r is primarily expressed by immune cells, as indicated by single-cell RNA-Seq datasets, and it is known to regulate hematopoietic proliferation and differentiation. We hypothesized that Csf1r overexpression in tumors might interfere with Csf1-mediated signals, leading to the reduced presence of immune cells observed in the tumors. To test this hypothesis, we generated Csf1r overexpressing mice. These showed accelerated tumor formation on a Trp53R270H mutant background and the tumors exhibited decreased levels of immune cells. Additionally, we found a strong correlation between CSF1 ligand, CSF1R, and overall immune transcript levels in human, canine, and murine osteosarcoma, except for the murine tumors that overexpress Csf1r.

Conclusion: In conclusion, our findings indicate that overexpression of Csf1r leads to the generation of osteosarcoma tumors with reduced immune presence. This is significant because previous studies have shown that reduced immune presence in osteosarcoma and other sarcoma types is associated with poor patient outcomes. By therapeutically increasing CSF1 levels within tumors, we may promote the proliferation and differentiation of immune cells, potentially improving patient outcomes. This approach offers advantages over personalized

therapies, as it may benefit all patients rather than just those with specific targets

(P 85) TARGETING CD99 ELICITS EWING SARCOMA CELL PHAGOCYTOSIS BY UNDIFFERENTIATED AND M2-LIKE MACROPHAGES AND INDUCES MACROPHAGE POLARIZATION TOWARD A M1-LIKE PHENOTYPE

Katia Scotlandi, PhD[1]; Cristina Manferdini, PhD[2]; Camilla Cristalli, PhD[2]; Gina Lisignoli, PhD[2]; Maria Cristina Manara, MD[3], (1) Istituto Ortopedico Rizzoli IOR IRCCS, Laboratory of Experimental Oncology, Bologna, Italy., Bologna, Emilia-Romagna, Italy, (2) IRCCS Istituto Ortopedico Rizzoli, Bologna, Emilia-Romagna, Italy, (3) IRCCS, Istituto Ortopedico Rizzoli, Laboratory of Experimental Oncology, Bologna Italy, Bologna, Emilia-Romagna, Italy

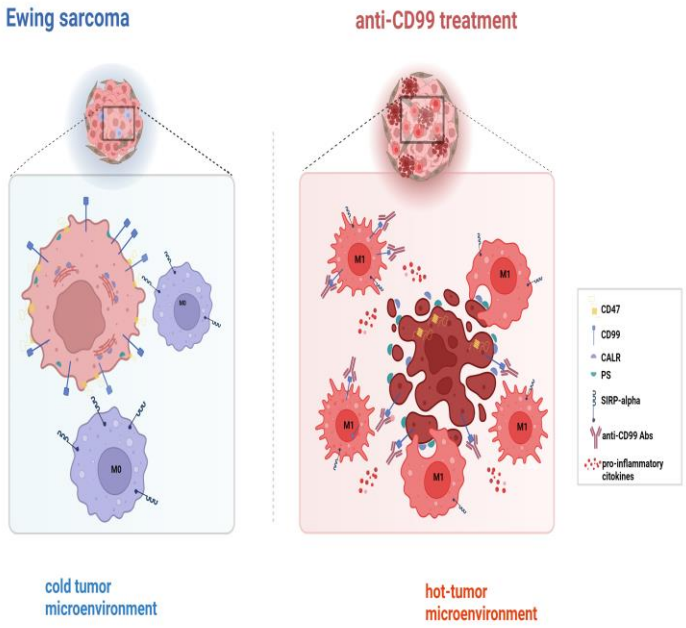
Objective: Tissue microenvironment affects the functional states of cancer cells and their response to treatments but exploiting it for therapy has remained a challenge. In the tumor microenvironment of Ewing sarcoma (EWS), the second most common pediatric bone tumor, macrophages are the most abundant immune cells and were found to be associated with worse patient's prognosis. In this study, we identified CD99, a hallmark of EWS cells that is also expressed on macrophages, as a novel relevant target that may be beneficial to improve immunotherapy of EWS.

Methods: Human M0 macrophages were differentiated ex vivo from circulating monocytes by culturing for seven days, checked for the expression of macrophage markers, and then used in a co-culture model to investigate the elimination of EWS cells treated or not with different anti-CD99 ABsbs or exosomes from CD99-genetically modified EWS cells: phagocytosis; time lapse-confocal microscopy; vital cell sounting were analyzed together with markers of M1-likevs M2-like phenotype (i.e.CD80, CD86, CD163, CD206, IL1β, IL6, IL10 and TNFα) by PCR and/or ELISA. Expression of eat-me and/or dont-eat me signals on EWS cells after anti-CD99 approaches was measured by cytoflorometry.EWS xenografts were treated with anti-CD99 Abs to check tumor growth and macrophage infiltration.

Results: Targeting CD99 on EWS cells downregulates the expression of the "don't eat-me" CD47 molecule while increasing the exposure of the "eat.me" phospahtidylserine and calreticulin molecules on the outer leaflet of tumor cell membrane and it triggers the engulfment and digestion of EWS cells by macrophages. In addition, CD99 ligation can result in the re-programming of undifferentiated M0 macrophages, or M2-like macrophage toward the inflammatory M1-like phenotype. This was confirmed also in vivo and the final result is a a significant inhibition of EWS tumor growth. In addition, experiments with exosomes modified for CD99 expression are ongoing.to verify the effects on tumor and macrophage recipient cells.

EWS xenografts treated with anti-CD99 Abs confirmed the recruiment of M1-like macrophages and inhibition of growth

Conclusion: This work reveals a previously unrecognized function for CD99,. which induces tumor cell phagocytosis and re-programs of macrophages, thus suggesting that approaches against CD99 may be exploited to improve immunotherapeutic strategies in EWS.



(P 86) DECIPHERING CELL-MATRIX INTERACTIONS IN OSTEOSARCOMA RESPONSE TO CHEMOTHERAPY IN A 3D TISSUE-ENGINEERED IN VITRO MODEL

Rameshwar R. Rao, MD Ph.D[1]; Michelle S. Huang, B.S.[2]; Sarah C. Heilshorn, PhD[2], (1) Stanford University, San Francisco, CA, (2) Stanford University, Stanford, CA

Objective: Osteosarcoma (OS), the most prevalent bone tumor diagnosed in the pediatric population, presents a grim 5-year survival rate of 60% in primary disease and a mere 20% in refractory and metastatic disease. Gold-standard chemotherapy was first introduced in the 1970's and involves multiple cycles of doxorubicin, cisplatin, and methotrexate. However, despite efforts in creating molecular-targeted drugs and immune-based cellular therapies, treatment regimens and survival rates remain unchanged. One major hurdle hindering the advancement of novel therapeutics is the lack of representative models that recapitulate the extracellular matrix (ECM) of the tumor. The intricate structural and signaling components of the ECM play a pivotal role in driving OS growth, development, and influencing response to chemotherapy.

Methods: We engineered a 3D tissue-engineered model for OS to investigate the effect of ECM on OS function, as well as the influence of cell-matrix interactions in determining the response to chemotherapy. The 143B OS cell line was encapsulated within hydrogels composed of collagen type 1, the primary ECM of bone tissue, through a pH and thermo-sensitive reaction. Traditional 2D culture on tissue culture plastic served as a control. Cell viability and cell morphology after 7 days of culture were assessed through Live/Dead staining. Cell phenotype was characterized through the expression of alkaline phosphatase activity and cell proliferation markers. Response to chemotherapy was quantified by metabolic activity and the organization of cytoskeletal proteins.

Results: OS cells exhibited high viability (>90%) in both 2D and 3D culture conditions but

altered their morphology to become more elongated and clustered into multicellular units in 3D, while cells remained rounded and dispersed in 2D (Fig. 1). Alkaline phosphatase, a biomarker of OS that is elevated at diagnosis and correlated with poor prognosis, was significantly increased in 3D compared to 2D at days 1, 3, and 7 in culture (Fig. 2). 2D cultures and 3D tumoroids were then grown for 7 days and exposed to chemotherapy for 24 hours followed by assessment of cellular metabolic activity to evaluate chemosensitivity. After exposure to doxorubicin, cisplatin, or a combination of doxorubicin and cisplatin, cellular metabolic activity was significantly higher in 3D tumoroids compared to 2D cultures (Fig. 3), suggesting that the cell phenotype in 2D is more chemosensitive, while the cell phenotype in 3D is more chemoresistant. As chemotherapy attacks the rapidly dividing cells in the body, we assessed for expression of Ki67, a nuclear protein, that is present in the active phases of the cell cycle (G1, S, G2) and is used as a marker of cell proliferation. Ki67 expression was significantly higher in 3D compared to 2D indicating that cells were actively proliferating at the time of chemotherapy exposure. We hypothesized that targeting the cytoskeletal microtubules of these elongated cells in 3D would rescue chemosensitivity, as these structures play a critical role in chromosome segregation during mitosis. To test this hypothesis, we added vinorelbine, a microtubule destabilizing agent used as chemotherapy for non-small cell lung cancer, to our 3D OS cultures. Immunocytochemistry confirmed the degradation of microtubules and significant alteration of the cell cytoskeleton along with a significant decrease in metabolic activity for the combinatorial therapy compared to doxorubicin and cisplatin alone (Fig. 4).

Conclusion: These data validate the need to study OS cells within a 3D ECM of collagen to capture cell-matrix interactions that are critical to the native tumor microenvironment. Our data suggest that combinatorial treatment with traditional front-line chemotherapy and a microtubule destabilizing agent may be beneficial. Future work will delve into the underlying molecular mechanisms governing OS cell-ECM interactions. While further exploration with patient-derived cell lines and patient-derived xenografts is necessary to validate the clinical relevance of this model, these results strongly support the use of 3D tissue engineered models as a valuable culture system for studying OS.

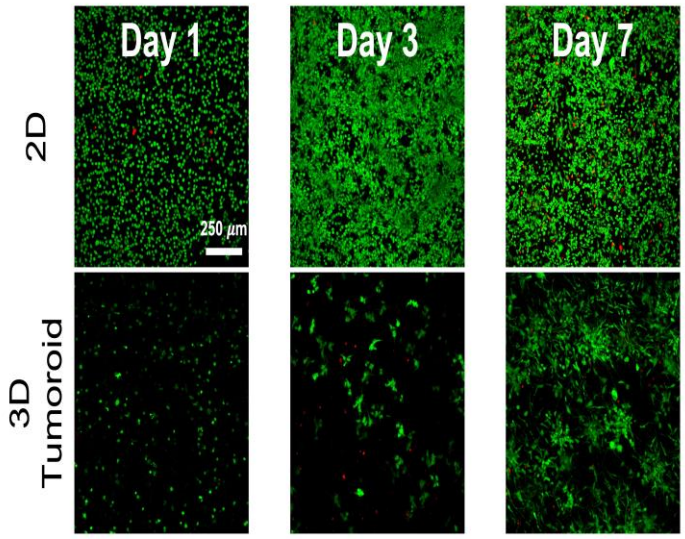


Figure 1. Cell viability of osteosarcoma cells in 2D compared to 3D tumoroid culture. Osteosarcoma cells embedded within 3D matrices exhibited high cell viability at Days 1, 3, and 7. OS cells exhibited a significant alteration in their morphology in 3D compared to 2D, whereby cells clustered and elongated into a spread phenotype in 3D.

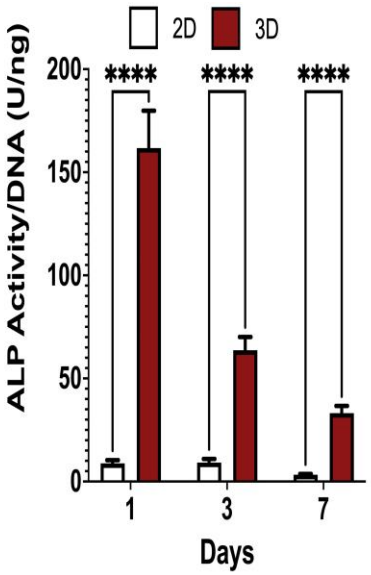


Figure 2. Alkaline Phosphatase Activity. Evaluation of alkaline phosphatase, the biomarker for osteosarcoma, at Days 1, 3, and 7 of culture demonstrated significantly higher intracellular expression in 3D tumoroids compared to 2D.

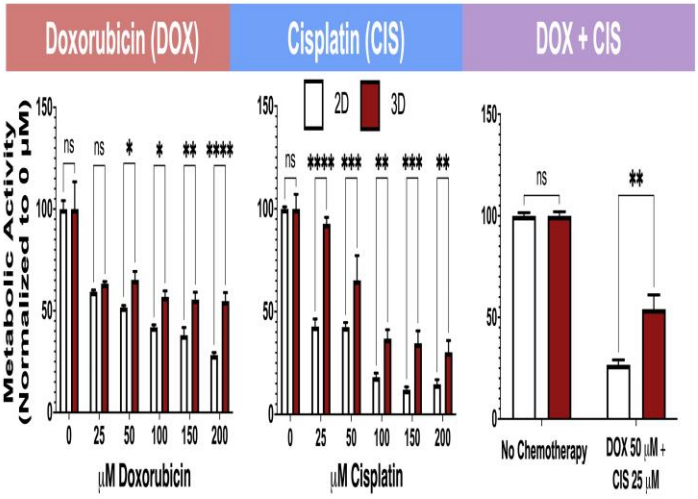


Figure 3. Response to chemotherapy. Osteosarcoma cells were cultured for 7 days in 3D tumoroids and 2D monolayer and then exposed to chemotherapy for 24 hours. Cells embedded within 3D tumoroids exhibited higher metabolic activity compared to 2D culture indicating that cell-matrix interactions play a pivotal role in the development of decreased chemosensitivity.

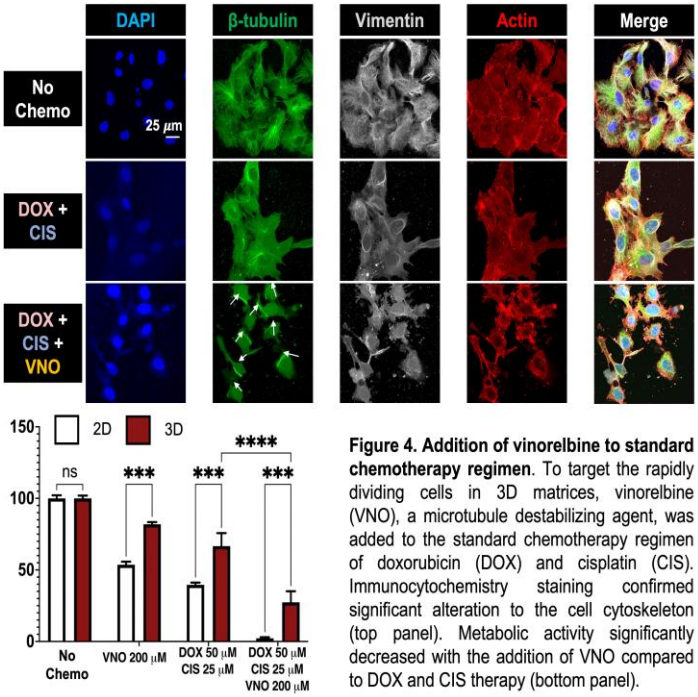


Figure 4. Addition of vinorelbine to standard chemotherapy regimen. To target the rapidly dividing cells in 3D matrices, vinorelbine (VNO), a microtubule destabilizing agent, was added to the standard chemotherapy regimen of doxorubicin (DOX) and cisplatin (CIS). Immunocytochemistry staining confirmed significant alteration to the cell cytoskeleton (top panel). Metabolic activity significantly decreased with the addition of VNO compared to DOX and CIS therapy (bottom panel).

(P 87) ANGIOSARCOMA OF THE ILIUM TREATED WITH HEMIPELVECTOMY AND HIP TRANSPOSITION FOLLOWED BY POSTOPERATIVE CHEMOTHERAPY WITH PAZOPANIB: A CASE REPORT

Yuki Aibara, MD[1]; Ukei Anazawa, MD, PhD[1]; Hiraku Hotta, MD, PhD[1]; Ryoma Aoyama, MD, PhD[1]; Satoshi Kamio, MD[2]; Toshiyuki Ogata, MD[1]; Aya Sasaki, MD, PhD[1]; Itsuo Watanabe, MD[1], (1) Tokyo Dental College Ichikawa General Hospital, Ichikawa, Chiba, Japan, (2) Tochigi Cancer Center, Utsunomiya, Tochigi, Japan

Objective: Primary angiosarcoma of bone is an extremely rare tumor, accounting for less than 1% of all malignant bone tumors, and cases in the pelvis represent only 15% of these tumors. Therefore, evidence regarding the efficacy of chemotherapy for advanced bone angiosarcoma is limited, and it is difficult to maintain progression-free disease. On the other hand, hip transposition has emerged as an alternative surgical technique for the reconstruction of the hip joint after hemipelvectomy. The objective of this study is to report a case of bone angiosarcoma with metastases, showing two years of survival. The treatment involved a hemipelvectomy and hip transposition using the Leeds-Keio (L-K) artificial ligament, followed by postoperative chemotherapy with pazopanib.

Methods: A case: A 69-year-old man presented with a history of right hip pain lasting for 18 months. Radiological findings indicated a pelvic tumor-like lesion, prompting the patient's referral to our hospital. He complained of hip pain and gait disturbance. The plain X-ray showed a lytic bone defect in the right iliac bone. CT image revealed destruction of the bony cortex of the ilium and a large mass extending beyond the ilium and into retroperitoneal space. CT angiography depicted rich feeding arteries of the tumor. On MRI, T1-weighted image revealed a heterogeneous mass with areas of low and partially high signal intensity, while the T2-weighted

image showed high signal intensity. PET-CT revealed multiple uptakes indicative of bone metastases in the bilateral humerus, left clavicle, right scapula, mandible, lumbar spine, sacral vertebrae, and ilium. However, no obvious lesion was observed other than bone. A CT-guided bone biopsy was performed, and the pathological diagnosis was a vascular malignant tumor. He complained of intractable right hip pain and was unable to walk. Due to the difficulty in pain control, surgical intervention for the iliac lesion was considered. Preoperatively, tumor embolization was performed to control bleeding. The tumor was excised, including the pubic bone, acetabular bone and sacroiliac joint with the iliopsoas muscle and the femoral nerve. After tumor resection, hip transposition was performed, in which bone tunnels were created in the femoral head and sacrum, respectively, and they were connected using the L-K artificial ligament. The pathological diagnosis was angiosarcoma.

Results: After surgery, the patient experienced pain relief in the hip joint and became able to walk with full weight bearing 8 weeks later. Postoperative chemotherapy was performed for multiple bone metastases. Because the patient could not visit the hospital frequently, pazopanib was administered 10 weeks after surgery. Ten months after surgery, the patient complained of right hip pain and MRI identified local recurrence around the right femur, which was treated with radiation therapy. Two years after surgery, brain and lung metastases developed, and the patient died of the disease 2 years and 2 months after surgery.

Conclusion: In this case of pelvic angiosarcoma, hemipelvectomy and hip transposition using an artificial ligament proved effective for restoring ambulatory ability. The survival period of M1 patients with angiosarcoma of bone was reported almost shorter than 1 year. Reported adjuvant regimens for them included paclitaxel, doxorubicin-ifosfamide, gemcitabine, etc. However, the efficacy of pazopanib, which has been reported only in advanced soft tissue angiosarcoma, remains uncertain for angiosarcoma of bone. In our case, the metastases were controlled for over 2 years after postoperative pazopanib administration. This suggested that pazopanib may be effective to bone angiogenic sarcoma.

Withdrawn ~~(P 88) PROCOLLAGEN C-PROTEIN ENHANCER 1 (PCPE-1) AS A NOVEL SERUM BIOMARKER FOR OSTEOSARCOMA~~

(P 89) GENERATION OF CHONDROCYTE-DERIVED MOUSE OSTEOSARCOMA MODELS - TRACKABLE SYSTEMS TO DELINEATE ONCOGENIC AND METASTATIC ENVENTS PRIOR TO TUMOROGENESIS

Xin Zhou, PhD[1]; Zhaohui xu, BS[2]; Zhongting Zhang, MS[3]; Hannah Beird, PhD[4]; Yanhua Yi, BS[5]; Wendong Zhang, BS[1]; Yifei Wang, MD[3]; Sylvester Jusu, PhD[5]; Michael Roth, MD[3]; Jonathan Gill, MD[1]; Richard Gorlick, MD[1], (1) The University of Texas MD Anderson Cancer Center, Houston, TX, (2) UT MD anderson cancer center, Houston, TX, (3) UT MD Anderson Cancer Center, Houston, TX, (4) Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA, Houston, TX, (5) UT MD Anderson cancer center, Houston, TX

Objective: Osteosarcoma (OS) incidence peaks during puberty and coincides with rapid longitudinal bone growth. Primary OS is frequently localized at the growing ends of long bones formed by endochondral ossification, a process driven by growth plates comprised of chondrocytes, which themselves are a significant source of osteoblasts contributing to bone

formation (X Zhou et al PlosGen 2014, L Yang et al PNAS 2014). Given the temporal and spatial links, we propose that growth plate chondrocytes are the origin of osteosarcoma cells, and if proven, we aimed to delineate the primary oncogenic as well as the disseminating events leading to osteosarcoma genesis.

Methods: To explore growth plate chondrocytes as a potential OS cell of origin, we conditionally deleted p53 alleles alone, or p53 and Rb1 alleles specifically in chondrocytes through either Col10a1-Cre (hypertrophic chondrocytes) or Agc-CreERT2 (all chondrocytes)-driven recombination. These mutant mice also harbor a fluorescence reporter allele (ROSA26-Tomato), which is expressed upon Cre recombination, providing a visual tool for tracking the entire collection of mutant cells, including the Cre-active ancestries and their descendances from their origins to any subsequent disseminating sites. The derived mice were observed for tumor formation.

Results: The Col10p53flfl(tm) and [Col10p53flflRbfl+(tm)] mutants developed osteoblastic OS with 100% penetrance, the latter cohort with shortened latencies and increased metastases compared to that of the former. The tamoxifen-induced AgcCreERT2p53flfl(tm) and AgcCreERT2p53flflRbfl+(tm) mutants developed OS in an age dependent manner correlating with periods of growth: the penetrance was inversely correlated with the age of the mice at the time of tamoxifen treatment, from 100% at younger than 2-week-old to nearly zero after 2 months of age, In both of the Cre-driven models, the tumors occurred exclusively in endochondral bones, often in metaphases adjacent to growth plates. The WGS of the primary and metastatic tumors revealed cytogenetic complexity resembling human OS. The pulmonary metastases were detectable at euthanasia in ~10% of the Col10p53flflRbfl+tm and ~25% of the AgcCreERT2p53flflRbfl+tm mutant mice that developed OS. Fluorescence microscopy uncovered varied numbers of tm+ cell clusters with sizes below the visible limit (< 0.5mm: micro) dispersed throughout the lungs of mOS mice, which didn't present perceptible lung malignancies at autopsy. In comparison, large pulmonary diseases were present in nearly all mice who were permitted to survive longer through amputation of the primary OS site. The micro- tm+ cells were capable of robust sarcosphere-formation, as well as capability for osteo- and adipogenesis in vitro. The RNA-seq profiling of lung metastatic cells identified sets of differentially expressed genes like that of human OS lung metastases. A hierarchical relationship existed between control, primary tumors, micro-metastatic lung disease and bulk lung metastases. The data suggested that the micro-metastatic lung cells may yield clues as to events earlier in the process of dissemination. Additional work also demonstrated less genetically complex OS cells in the regional bone marrow stroma, suggesting early precursors exist in that environment as well.

Conclusion: The chondrocyte-derived murine OS resembles human OS in histology, cytogenetic complexity, expression profiles and drug responses. Compared to Osx-Cre-driven osteosarcoma models the skeletal distribution of the OS is more reflective of patterns seen in human OS. This is concordant with the known relationship of osteosarcoma to growth plates. The reporter transgene allowed us to detect, isolate and characterize micro-metastatic cells. These micro-metastatic cells may yield insights into key genetic pathways involved in metastases. OS precursor cells were present in the mutant bone stroma as well. We believe these chondrocyte-derived murine OS models are faithful tools with which to study OS derivation and dissemination.

(P 90) **RESULTS FROM AN INTERNATIONAL SURVEY OF BONE SARCOMA PATIENTS**

Pan Pantziarka, PhD[1]; Zoe Davison, BSc, PhD[2]; Natalia Fernandez, PhD[3]; Kathrin Schuster[4]; Angelika Sandakly, MBA[5]; Barbara C. Gonzalez-Vaughn, MA[6]; Jazzmin Huber, MBChB, BSc[7]; Caroline Kooy[8]; Ornella Gonzato, MD/MBA[9], (1) George Pantziarka TP53 Trust/Anticancer Fund, Kingston upon Thames, England, United Kingdom, (2) Bone Cancer Research Trust, Leeds, England, United Kingdom, (3) SPAGN Sarcoma Patient Advocacy Global Network, Barcelona, Catalonia, Spain, (4) Sarcoma Patient Advocacy Global Network (SPAGN), Woelfersheim, Hessen, Germany, (5) InfoSarcomes France, Valbonne, Provence-Alpes-Cote d'Azur, France, (6) Sarkomer, Oslo, Oslo, Norway, (7) Myrovlytis Trust, London, England, United Kingdom, (8) Patientenplatform Sarcomen/Chordoma Foundation, Utrecht, Utrecht, Netherlands, (9) Fondazione Paola Gonzato- Rete Sarcoma ETS, Udine, Friuli-Venezia Giulia, Italy

Objective: To create a comprehensive landscape of the views and experiences of the global bone sarcoma patient community in order to provide input to clinical delivery and help set research agendas. Further, to explore commonalities and differences between the different bone sarcoma types from the perspective of patients. Arising from discussions in the patient and parent group within the FOSTER (Fight OsteoSarcoma Through European Research) consortium a group of patients, parents and advocates from multiple countries and bone sarcoma patient communities have come together to produce an international online survey.

Methods: Available in six languages, this online survey asks questions on diagnosis and treatment, priorities for clinical research, quality of life and survivorship. The survey is designed to be global in scope rather than European only, as well as including all bone sarcoma types. In addition to demographic data, such as age, gender, country and disease type, the data shows the priorities for future research broken down by disease (Chordoma/Osteosarcoma/Ewing's sarcoma/Chondrosarcoma/Other).

Results: The survey was launched in January 2023. An interim analysis in May 2023 showed that detailed data from 480 respondents, from 23 countries, had been collected. In terms of disease areas, data had been collected for Chordoma (n=70, 15%), Osteosarcoma (n=173, 36%), Ewing's sarcoma (n=100, 21%), Chondrosarcoma (n=47, 10%) and other bone sarcomas (n=31, 6%). Demographic data, in terms of age and gender distribution for these responses are in line with clinical data. For example, median age, in years, at diagnosis by disease type is Chordoma 47.5, Osteosarcoma 15, Ewing's sarcoma 14, Chondrosarcoma 41 and other 39. While the majority of patients in all subtypes are diagnosed with localised disease (60 – 70%), the rate of initial misdiagnosis varies by sarcoma type - Chordoma 35%, Osteosarcoma 21%, Ewing's sarcoma 28%, Chondrosarcoma 44%, and other 54%. The top three ranked research priorities in diagnosis, drug treatment, surgery, support needs, survivorship and quality of life research also show a diverse pattern of overlaps and differences according to bone sarcoma type.

Conclusion: The survey will remain open until December 2023, and two more languages are due to be added. Analysis of the latest data will be performed prior to CTOS Dublin, including more detailed exploration of patient experience of current treatments and priorities for future research. Our preliminary data already shows that although the different bone sarcoma types differ in biology and treatment, there are also many commonalities from a patient perspective. Results to date show remarkable convergence of ranked priorities across the different bone sarcomas, providing the opportunity for potential synergies in research. In addition, the experience of bringing together the different bone sarcoma subtypes has been positive and a major driver of the emergence of a distinct bone sarcoma community ready to collaborate

further.

Age (years) at diagnosis by disease

	Median Age	SD
Chordoma	47.5	16.2
Osteosarcoma	15.0	14.4
Ewing's sarcoma	14.0	10.2
Chondrosarcoma	41.0	14.6
Other	39.0	17.7

Demographics

	Responses	Male(% of total)	Female(% of total)	Patient(% of total)	Parent(% of total)	Other(% of total)
English	92	35 (7.3%)	57 (11.9%)	54 (11.3%)	24 (5%)	14 (2.9%)
Spanish	33	16 (3.3%)	17 (3.5%)	12 (2.5%)	8 (1.7%)	13 (2.7%)
Italian	151	72 (15%)	79 (16.5%)	49 (10.2%)	73 (15.2%)	29 (6%)
French	39	19 (4%)	20 (4.2%)	19 (4%)	19 (4%)	1 (0.2%)
German	69	21 (4.4%)	48 (10%)	31 (6.5%)	24 (5%)	14 (2.9%)
Dutch	96	50 (10.4%)	44 (9.2%)	55 (11.5%)	29 (6%)	12 (2.5%)
Total	480	213 (44.4%)	265 (55.2%)	220 (45.8%)	177 (36.9%)	83 (17.3%)

Responses by country

Country	Number
Argentina	1
Australia	1
Austria	2
Belgium	11
Bosnia and Herzegovina	1
Canada	3
Colombia	2
Ecuador	1
France	41
Germany	67
Ireland	8
Israel	1
Italy	150
Morocco	1
Netherlands	81
Norway	1
Poland	1
Romania	1
South Africa	1
Spain	30
Sweden	3
United Kingdom	43
United States	24
Not specified	5
Total	480

(P 91) BLOOD TRANSFUSION AND SURVIVAL AMONG CHILD, ADOLESCENT, AND YOUNG ADULT PATIENTS WITH OSTEOSARCOMA: A RETROSPECTIVE COHORT STUDY

Sukjoo Cho, MD[1]; Jamie L. Fierstein, PhD[2]; Racha T. Khalaf, MD, MSCS[3]; John M. Morrison, MD, PhD[4]; Jonathan Metts, MD[5], (1) Department of Pediatrics, University of South Florida Morsani College of Medicine, Decatur, GA, (2) Epidemiology and Biostatistics Shared Resource, Johns Hopkins All Children's Institute for Clinical and Translational Research, Johns Hopkins All Children's Hospital, St. Petersburg, FL, (3) Department of Pediatrics, University of South Florida Morsani College of Medicine, Tampa, FL, (4) Division of Pediatric Hospital Medicine, Johns Hopkins All Children's Hospital, St. Petersburg, FL, (5) Moffitt Cancer Center and Johns Hopkins All Children's Hospital, Tampa, FL

Objective: Prior data suggest that pretransplant blood transfusion markedly increases survival of kidney allograft in a dose-dependent fashion, presumably by creating immune tolerance in the recipients. Such data has raised concerns that blood transfusion among oncologic patients may subdue anti-cancer immunity and thereby worsen survival outcomes. Many observational studies and meta-analyses reported that transfusion was associated with decreased survival and increased recurrence of various tumors including colorectal cancer and lung cancer. However, scarce data are available for pediatric malignancies. The primary objective of this study, therefore, is to investigate the association between post-diagnosis blood transfusion and survival of child, adolescent, and young adult (CAYA, defined as 39 years of age or younger) patients with osteosarcoma.

Methods: This retrospective cohort study included patients with osteosarcoma at our institution from 2007 to 2022. Search queries for osteosarcoma were built using eligibility criteria consisting of diagnostic codes for bone cancer (C40 or C41) and a medication code for cisplatin (2555) as per the International Classification of Diseases 10th Revision. Cisplatin is a standard-of-care first-line chemotherapy for osteosarcoma but rarely given for other bone sarcomas. Identified patients were manually reviewed to confirm the diagnosis of osteosarcoma and classified according to whether transfusion was required during the initial 10 weeks of chemotherapy. Kaplan-Meier estimates of 3- and 5-year overall survival (OS) and event-free survival (EFS) were calculated. Inter-group survival functions were compared with the log-rank test while point-wise comparisons were calculated using Z-tests.

Results: Among 79 CAYA patients identified with osteosarcoma, six were excluded due to unclear diagnosis date, comorbidity of Diamond-Blackfan anemia, and/or considerable treatment occurring at the outside facility. Of the remaining 73 patients, 34 (46.6%) required blood transfusion during the first 10 weeks of chemotherapy prior to surgery. Among transfused patients, 30 (88.2%) received red blood cells (RBCs) and 14 (41.2%) received platelets, while 10 (29.4%) received both platelets and RBCs. Table 1 describes demographic and clinical characteristics by transfusion status. Median (range) age at diagnosis for transfused and non-transfused patients was 15 (9 to 28) and 14 (4 to 29), respectively. Sex at birth was evenly distributed among the non-transfused group while the majority of the transfused group was male (74.4%). There were no significant inter-group differences in race and ethnicity, primary tumor location, stage, or exposure to ifosfamide. Median (range) survival time for the entire cohort was 2.9 (0.5 to 11.4) years. Among the transfused group, median (range) survival was 3.8 (0.8 to 11.4) years and among the non-transfused group, median (range) survival was 2.8 (0.5 to 6.4) years. Table 2 & 3 and Figure 1 summarize OS and EFS of patients by transfusion type and status. As seen in Table 2, there were no observed inter-group differences in OS and

EFS at 3- (OS: 81.2% vs. 81.0%, p>.9; EFS: 74.9% vs. 73.6%, p=.9) or 5-years (OS: 76.7% vs. 70.8%, p=.6; EFS: 58.2% vs. 41.7%, p=.3): Three-year EFS of patients not transfused with platelets differed significantly from patients transfused with platelets (70% vs. 91.7%, p=.04). There were no other significant associations observed between 3- and 5-year EFS or OS and RBC or platelet transfusion.

Conclusion: These data did not demonstrate a significant association between blood transfusion and OS or EFS of CAYA patients with osteosarcoma. Given the small sample size and retrospective nature of the study, further investigation with a larger, prospective cohort should be considered.

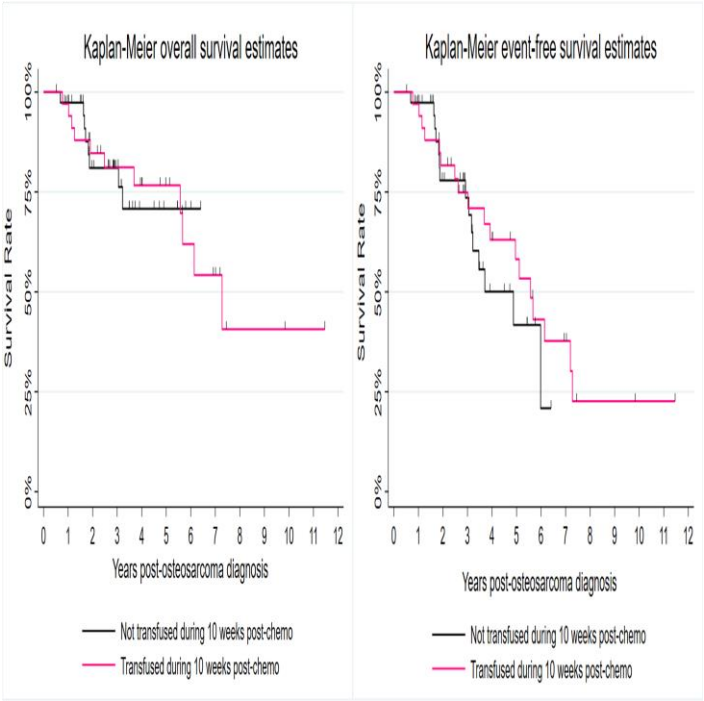
Characteristics	Transfusion (n=34)	No transfusion (n=39)	Total (n=73)	P-value
Sex, n(%)				
Male	16 (47.1)	29 (74.4)	45 (61.6)	.02
Female	18 (52.9)	10 (25.6)	28 (38.4)	
Race, n(%)				
Black or African American	5 (14.7)	7 (18.0)	12 (16.4)	.4
White	27 (79.4)	23 (59.0)	50 (68.5)	
Asian	1(2.9)	2 (5.1)	3 (4.1)	
Native Hawaiian or Other Pacific Islander	0(0)	1 (2.6)	1 (1.4)	
Multiracial	0 (0)	2 (5.1)	2 (2.7)	
Unknown/not reported	1(2.9)	2 (5.1)	3 (4.1)	
Ethnicity, n(%)				
Hispanic/Latino	4 (11.8)	8 (20.5)	12 (16.4)	.5
Non-Hispanic/Latino	18 (52.9)	21 (53.9)	39 (53.4)	
Unknown/not reported	12 (35.3)	10 (25.6)	22 (30.1)	
Ifosfamide as first-line chemotherapy, n(%)				
Yes	3 (8.8)	1 (2.6)	4 (5.5)	.3
No	31 (91.2)	38 (97.4)	69 (94.5)	
Radiation, n(%)				
Yes	9 (26.5)	8 (20.5)	17 (23.3)	.5
No	25 (73.5)	31 (79.5)	56 (76.7)	
Stage at osteosarcoma diagnosis, n(%)				
Localized	26 (76.5)	33 (84.6)	59 (80.8)	.4
Metastatic	8 (23.5)	6 (15.4)	14 (19.2)	
Location of osteosarcoma, n(%)				
Extremity	31 (91.2)	37 (97.9)	68 (93.2)	.5
Non-extremity	3 (8.8)	2 (5.1)	5 (6.9)	
Secondary malignancy, n(%)				
Yes	0 (0)	2 (5.1)	2 (2.7)	.5
No	34 (100)	37 (94.9)	71 (97.3)	
Age at osteosarcoma diagnosis				
N	34	39	73	.5
Median	14	15	15	
Range (minimum to maximum)	4 to 29	9 to 28	4 to 29	

Survival rates	Transfusion (n=34)	No transfusion (n=39)	P-value	Total
OS (95% CI)				
3-year OS	81.2% (62.7% to 91.1%)	81.0% (62.4% to 91%)	>.9	81.0% (68.9% to 88.8%)
5-year OS	76.7% (56.6% to 88.3%)	70.8% (48.7% to 84.7%)	.6	75.7% (61.7% to 85.1%)
Median OS	3.8	2.8		2.9
Range (minimum to maximum)	0.8 to 11.4	0.5 to 6.4		0.5 to 11.4
EFS (95% CI)				
3-year EFS	74.9% (55.9% to 86.6%)	73.6% (53.6% to 86%)	.9	74.1% (61.0% to 83.3%)
5-year EFS	58.2% (37.5% to 74.2%)	41.7% (19.7% to 62.5%)	.3	52.2% (36.9% to 65.5%)

OS, overall survival; EFS, event free survival; RBC, red blood cell

OS rate (95% CI)	RBC transfusion (n=30)	No RBC transfusion (n=43)	P-value	Platelet transfusion (n=14)	No platelet transfusion (n=59)	P-value
3-year OS	79.4% (59.7% to 90.2%)	82.6% (65.2% to 91.8%)	.7	100%	76.8% (62.6% to 86.2%)	-
5-year OS	74.5% (53.2% to 87.1%)	73.4% (52.6% to 86.1%)	.9	90% (47.3% to 98.5%)	70.4% (54.3% to 81.7%)	.1
EFS rate (95% CI)	**RBC transfusion (n=30)**	**No RBC transfusion (n=43)**	**P-value**	**Platelet transfusion (n=14)**	**No platelet transfusion (n=59)**	**P-value**
3-year EFS	75.6% (55.5% to 87.6%)	73.1% (54.3% to 85.1%)	.8	91.7% (53.9% to 98.8%)	70% (55.0% to 81.0%)	.04
5-year EFS	62.3% (40.7% to 77.9%)	37.5% (16.6% to 58.4%)	.09	61.1% (25.5% to 83.8%)	49.1% (32.4% to 63.9%)	.5

OS, overall survival; EFS, event free survival; RBC, red blood cell



(P 92) DOSIMETRIC CHARACTERISTICS, OUTCOMES AND PROGNOSTIC FACTORS OF PATIENTS WITH EXTRACRANIAL CHORDOMAS AND CHONDROSARCOMAS TREATED WITH HIGH DOSE RADIOTHERAPY: A LARGE SINGLE INSTITUTION COHORT

Adam Mitchell, MBChB MRes MRCP FRCR[1]; Mahbubl Ahmed, MRCP PhD FRCR[1]; Franel Le Grange, MBChB FCRadOnc MD[2]; Avinash Pilar, MBBS MD FRCR[1]; Beatrice Seddon, MBBS BSc MRCP FRCR PhD[1]; Pei Lim, n/a[3], (1) University College London Hospital, London, England, United Kingdom, (2) UCLH, London, England, United Kingdom, (3) University College Hospitals London, London, England, United Kingdom

Objective: Chordomas (Ch) and chondrosarcomas (CS) are radio-resistant tumours requiring high dose radiotherapy (RT) for local control. Most published studies describe prescribed RT doses which could be different to received doses in the final RT plan due to compromises required for critical organs and implants.

Methods: We retrospectively analysed 71 adult patients from University College London Hospitals who were treated with high-dose (>45Gy EqD2) proton or photon RT for extracranial Ch and CS between 2002 and October 2017. Electronic medical records were reviewed and RT data, including prescribed and received doses, were extracted. Patients with extra-skeletal mesenchymal CS and skull base tumours were excluded. This study was approved as a service evaluation with the local radiotherapy governance directorate at UCLH.

Median age was 59 years (range 22-85 years). Median follow-up duration was 42 months (range 4-121 months). RT modalities used were protons (PBT) only (31.0%), mixed PBT/Intensity Modulated RT (IMRT) (12.7%), IMRT (23.9%) and 3D-Conformal RT (3D-CRT) (32.4%). More patients received PBT, including mixed plans, for Ch (50%, n=31) compared with

CS (34.5%, n=10). 68 patients (95.8%) received RT to the primary site of disease, of which 75% (n=51) were treated at the initial presentation and 25% (n=17) at recurrence. 43.7% (n=31) of patients had macroscopic residual disease, and 18.3% (n=13) had metastases at the time of RT. Sites treated included: spine (46.5%), sacrum (40.9%), extremity (5.6%), paraspinal (4.2%) and pelvis (2.8%). Further demographics are detailed in Table 1.

40 patients had two RT treatment dose levels prescribed, split into PTV low dose (LD) and high dose (HD) volumes. Otherwise, patients were considered to have PTV-HD level only.

Results: 30 (42.3%) local failures at 2-57 months were observed; 16 (55.2%) within the CS cohort and 14 (33.3%) within the Ch cohort. The 4-year local control (LC) rate was 47.2%; CS 37.5% and Ch 54.5%.

On univariate analysis, LC was significantly worse ($p \leq 0.05$) for: CS histology, poorly differentiated/de-differentiated subtype, tumour diameter □5cm, pelvic anatomical site, local recurrence prior to RT and presence of macroscopic disease at time of RT. There was a trend towards improved LC if received PTV-HD D95% >65Gy was achieved (Fig 1).

Overall survival (OS) and distant progression free survival (DPFS) analyses were performed for the non-metastatic cohort (n=58). 4-year OS was 66.8%, with CS at 57.4% and Ch at 72.1%. 4-year estimated DPFS was 64.6% and 80.8% for CS and Ch, respectively. OS was significantly worse if local ($p < 0.001$) or distant ($p < 0.002$) recurrence occurred post RT.

RT was well tolerated, with 4 patients (5.6%) experiencing >Grade 2 acute and late toxicity.

Median PTV-HD volume was 516.4cc (range 63.5-7849.9cc) for CS and 456.6cc (65.3-3025.6cc) for Ch. Received dose PTV-HD V95% was 94.9% for CS and 94% for Ch. Median prescribed and received PTV-HD D95% were similar for most modalities, except the mixed PBT/IMRT group (11.6Gy difference) (Fig 2). High PTV-HD D95% doses were achieved for IMRT plans in the Ch group (66.7Gy) but not in the CS group (44.1Gy) (Table 1). This is likely due to the anatomical distribution differences observed; all extremity sites were in the CS group.

Larger PTV-HD volume and lower received PTV-HD D95% were associated with poorer LC when assessed as continuous variables. Similar LC was observed for patients treated with IMRT or PBT only modality but a trend towards worse LC was observed with 3D-CRT and mixed PBT/IMRT technique (Fig 3). This could be explained by choice of mixed plans for patients with metalwork in situ.

Conclusion: High dose RT for Ch and CS was well tolerated and effective. Similar LC was observed with IMRT or PBT-only techniques. High dose dosimetry to PTV-HD volumes were achieved for Ch with IMRT plans, suggesting that advanced photon RT may remain an appropriate treatment option for patients unable to access PBT.

Table 1 – Patient Demographics

Factors		Chondrosarcoma (n=29)	% of CS	Chordoma (n=42)	% of chordoma	All (n=71)	% of total
Age at start of RT (years)	Median	49.4		64.5		58.6	
	Range	23.5-79.2		22.3-84.9		22.3-84.9	
Gender	Female	10	34.5	15	35.7	25	35.2
	Male	19	65.5	27	64.3	46	64.8
Histology subtype	Conventional	22	75.9	39	92.9	61	85.9
	Clear cell (CS)	3	10.3	0	0.0	3	4.2
	Poorly diff (Ch)	0	0.0	3	7.1	3	4.2
	Dedifferentiated	4	13.8	0	0.0	4	5.6
Grade	G1	5	17.2	39	92.9	58	81.7
	G2	14	48.3				
	High grade (G3, poor diff, clear cell)	6	20.7	3	7.1	9	12.7
	Dedifferentiated	4	13.8	0	0.0	4	5.6
Maximum dimension of tumour at diagnosis (cm)	Median	7.8		7.0		7.6	
	Range	3.3-15.0		2.0-34.0		2.0-34.0	
	Missing data	1		5		6	
Site of treatment	Sacrum	3	10.3	26	61.9	29	40.9
	Spine	17	58.6	16	38.1	33	46.5
	Pelvis	2	6.9	0	0.0	2	2.8
	Limb	4	13.8	0	0.0	4	5.6
	Paraspinal	3	10.3	0	0.0	3	4.2
Metastases at time of RT	Metastases	7	24.1	6	14.3	13	18.3
	No metastases	22	75.9	36	85.7	58	81.7
Disease treated at RT	Primary, First presentation	18	62.1	33	78.6	51	71.8
	Primary, Recurrence ^	9	31.0	8	19.0	17	24.0
	Metastases	2	6.9	1	2.4	3	4.2
RT technique	Proton	4	13.8	18	42.9	22	31.0
	Proton + IMRT	6	20.7	3	7.1	9	12.7
	IMRT	7	24.1	10	23.8	17	23.9
	3D CRT	12	41.4	11	26.2	23	32.4
Treatment intent at RT	Adjuvant	22	75.9	27	64.3	49	69.0
	Definitive^^	4	13.8	12	28.6	16	22.5
	Palliative	3	10.3	3	7.1	6	8.5
Disease status at time of RT	Clear	1	3.4	10	23.8	11	15.5
	Microscopic	16	55.2	13	30.2	29	40.8
	Gross	12	41.4	19	45.2	31	43.7
			Range		***Range***		***Range***
Median prescribed dose to PTV HD	Proton	68.4	66-70.1	71.1	63.3-81.6	70.1	63.3-81.6
	Proton + IMRT	70.1	68.4-70.1	70.1	70.1-70.1	70.1	68.4-70.1
	IMRT	60.0	50-66	70.0	47.9-78.8	63.3	47.9-78.8
	3D CRT	50.7	46-72	47.9	42.8-72	50.0	42.8-72
Median dose of received PTV HD D95% with plan type	Proton	60.7	52.3-66.5	69.2	49.9-79.2	67.0	49.9-79.2
	Proton + IMRT	58.4	50.8-66.3	58.5	55.1-62.3	58.5	50.8-66.3
	IMRT	44.1	0-62.7	66.7	45.6-74.1	61.6	0-74.1
	3D CRT	50.0	42-66	47.1	38.0-51.3	47.7	38.0-66.0

^This group includes patients in whom RT was indicated because of local recurrence as the presentation requiring RT, rather than those whose disease recurred between surgery + RT.
^^ All patients in the definitive group were prescribed EqD2 doses of more than 60Gy.

Figure 1 - Kaplan-Meier survival analysis demonstrating local control if planned HD-PTV D95% >65Gy, compared with 50-64.9Gy and <50Gy.

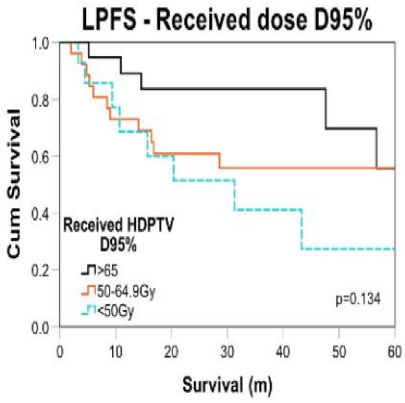


Figure 2 - Box plot of prescribed and received dose to high dose PTV, by radiotherapy technique used.

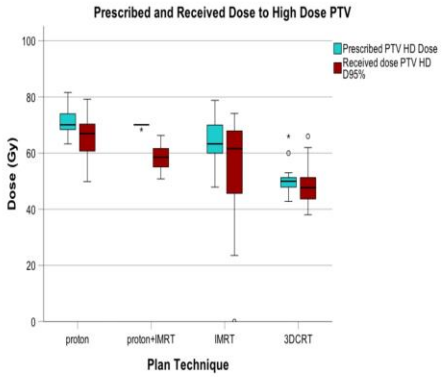
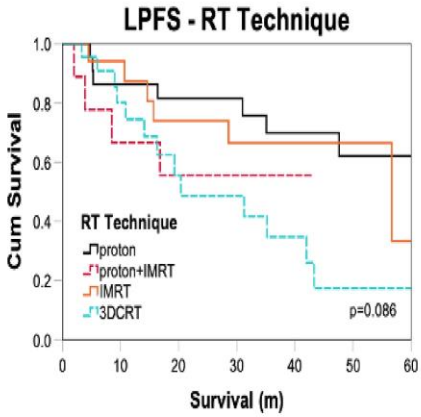


Figure 3 - Kaplan-Meier survival analysis demonstrating local control by radiotherapy technique used.



(P 93) INDETERMINATE CHEST LESIONS IN PATIENTS WITH BONE SARCOMA INCIDENTAL FINDING VERSUS METASTATIC DISEASE

Babe Westlake, DO[1]; John Groundland, MD[2]; Jeffrey Brown, MD[3]; Jacqueline Hart Tokson, BS[2]; Kevin B. Jones, MD[4], (1) Renown Regional Medical Center, Reno, NV, (2) Huntsman Cancer Institute, Salt Lake City, UT, (3) Huntsman Cancer Institute, Salt Lake, UT, (4) University of Utah, Salt Lake City, UT

Objective: To identify the frequency of benign pulmonary nodules versus true metastatic disease in adult patients with a bone sarcoma and to identify what factors predict the progression of indeterminate pulmonary nodules to true metastatic disease.

Methods: This was a retrospective cohort study of adult patients presenting with a bone sarcoma at a single institution from 2010-2020. Patients were excluded if they had known metastatic disease at time of diagnosis, underwent a non-oncologic surgery or had incomplete staging and surveillance imaging with CT scan. Demographic variables, oncologic variables (histology, grade) and variables associated with indeterminate nodules (size, number) both at presentation and follow up were collected. Descriptive statistics were used to summarize variables of interest and regression analyses performed to look at associations of progression of indeterminate nodules to metastatic disease versus those that did not.

Results: Less than half of patients that presented with indeterminate nodules progressed to metastatic disease (41%). Of patients that developed new nodules during surveillance 55% progressed to metastatic disease. Number and size of nodules at presentation was not associated with progression of nodules to metastatic disease.

Conclusion: Less than half of patients with indeterminate nodules at presentation will progress to metastatic disease and about half of patients that develop new nodules on surveillance will progress to metastatic disease.

Table 2. Patients presenting with primary sarcoma of bone without evident metastatic disease on initial staging but with indeterminant nodules on CT scan of the chest: Descriptive data of patients the sub-groups that did and did not develop metastatic disease, along with comparison between groups that developed metastatic disease vs those that did not.

		All Patients Presenting with Indeterminant Nodules on Initial CT Chest Scan	Patients who Progressed to Metastatic Disease	Patients Who Did Not Progress to Metastatic Disease	p value*
n		73	30	43	-
Age (years)		50	50.9 (mean)	49.5 (mean)	0.814
Follow-Up (months)		40.9	22.9 (mean)	53.7 (mean)	<0.001
Gender	Male	41	16 (39.0%)	25 (61.0%)	0.166
	Female	32	14 (43.8%)	18 (56.3%)	
Smoking History	Never	42	17 (40.5%)	25 (59.5%)	0.216
	+Smoking history	25	10 (40.0%)	15 (60.0%)	
	Unknown	6	3 (50.0%)	3 (50.0%)	
Diagnosis	Osteosarcoma	24	9 (37.5%)	15 (62.5%)	0.637
	Chondrosarcoma	30	12 (40.0%)	18 (60.0%)	
	Ewing Sarcoma	4	2 (50.0%)	2 (50.0%)	
	Chordoma	2	0 (0.0%)	2 (100.0%)	
	Other	13	7 (53.8%)	6 (46.2%)	
Grade	Low	5	1 (20.0%)	4 (80.0%)	0.321
	High	68	29 (42.6%)	39 (57.4%)	
Anatomic Location	Upper Extremity	10	3 (30.0%)	7 (70.0%)	0.805
	Lower Extremity	32	13 (40.6%)	19 (59.4%)	
	Spine/Chest Wall	8	3 (37.5%)	5 (62.5%)	
	Pelvis	23	11 (47.8%)	12 (52.2%)	
Size of Largest Indeterminate Nodules**	1-5 mm	57	22 (38.6%)	35 (61.4%)	0.615
	6-9 mm	11	6 (54.5%)	5 (45.5%)	
	10+ mm	5	2 (40.0%)	3 (60.0%)	
Number of Indeterminate Nodules	1	20	7 (35.0%)	13 (65.0%)	0.712
	2	21	8 (38.1%)	13 (61.9%)	
	3	18	8 (44.4%)	10 (55.6%)	
	4	6	4 (66.7%)	2 (33.3%)	
	5+	8	3 (37.5%)	5 (62.5%)	

Table 4. Patients presenting with primary sarcoma of bone without evident metastatic disease who subsequently developed indeterminant nodules on CT scan of the chest: comparison between the sub-group that developed metastatic disease vs those that did not.

		Patients Who Developed Indeterminant Nodules on Follow Up CT Scans of the Chest Who Then Developed Metastatic Disease	Patients Who Developed Indeterminant Nodules on Follow Up CT Scans of the Chest But Did Not Develop Metastatic Disease	p value*
n		32	26	
Age (years)		48.3	51.1	0.622
Follow-Up (months)		37.3	45.5	0.579
Gender	Male	19 (55.9%)	15 (44.1%)	0.897
	Female	13 (54.2%)	11 (45.8%)	
Smoking History	Never	21 (55.3%)	17 (44.7%)	0.967
	+Smoking history	8 (53.3%)	7 (46.7%)	
	Unknown	3 (60.0%)	2 (40.0%)	
Diagnosis	Osteosarcoma	8 (57.1%)	6 (42.9%)	0.761
	Chondrosarcoma	11 (47.8%)	12 (52.2%)	
	Ewing Sarcoma	4 (80.0%)	1 (20.0%)	
	Chordoma	2 (50.0%)	2 (50.0%)	
	Other	7 (58.3%)	5 (41.7%)	
Grade	Low	1 (33.3%)	2 (66.7%)	0.435
	High	31 (56.4%)	24 (43.6%)	
Anatomic Location	Upper Extremity	3 (27.3%)	8 (72.7%)	0.219
	Lower Extremity	13 (59.1%)	9 (40.9%)	
	Spine/Chest Wall	3 (60.0%)	2 (40.0%)	
	Pelvis	13 (65.0%)	7 (35.0%)	
Presence of Indeterminant Nodules on Initial/ Pre-Operative CT Chest	Yes	13 (52.0%)	12 (48.0%)	0.672
	No	19 (57.6%)	14 (42.4%)	
Size of Largest Nodule on Initial/ Pre-Operative CT Chest	1-5 mm	12 (60.0%)	8 (40.0%)	0.347
	6-9 mm	0 (0%)	2 (100%)	
	10+ mm	1 (33.3%)	2 (67.7%)	

(P 94) DEVELOPING NOVEL OLIGONUCLEOTIDE THERAPIES TO MODULATE INSULIN RECEPTOR ALTERNATIVE SPLICING IN PEDIATRIC BONE SARCOMAS

Akila S. Venkataramany, BA[1]; Safiya Khurshid, PhD[2]; Matias Montes, PhD[3]; Pin-Yi Wang, PhD[2]; Dawn Chandler, PhD[2]; Timothy P. Cripe, MD, PhD[2], (1) Nationwide Children's Hospital / The Ohio State University College of Medicine, Columbus, OH, (2) Nationwide Children's Hospital, Columbus, OH, (3) Stanford University, Stanford, CA

Objective: Despite multifaceted treatment approaches for pediatric bone sarcomas, cancer recurrence and relapse are common, and survival outcomes for these young patients are as low as 25%. Both osteosarcoma (OS) and Ewing sarcoma (ES) have harnessed the IGF-I receptor (IGF-IR) and the IGF/insulin receptor (IR) signaling pathways for their progression and spread. Initial anti-IGF-IR therapies like figitumumab were unsuccessful in a majority of patients in part due to the IR, which can bind the same ligands as IGF-IR and potentiate downstream signaling despite IGF-IR inhibition. IR has two alternatively spliced isoforms—IR-A (exon 11 skipped) and IR-B (exon 11 included). Increased expression of IR-A, an isoform frequently expressed in cancer, may effectively evade current therapeutic mechanisms and contribute to resistance in patients. Our previous work illustrates the development of a splice-switching oligonucleotide (SSO) that significantly shifts IR splicing toward IR-B and slows rhabdomyosarcoma proliferation by targeting the binding sequence of CUG-BP1, a negative splicing regulator upstream of exon 11 (PMID: 35017650). We sought to evaluate the efficacy of this SSO in OS and ES cell lines and patient-derived xenografts (PDX). We also focused on improving in vitro and in vivo delivery of our SSO by expressing it via an adeno-associated viral (AAV) vector.

Methods: We used standard PCR protocols to assess IR isoform expression in a panel of OS and ES cell lines and PDXs at baseline and upon 24 hour SSO transfection with Lipofectamine 3000. We measured OS cell proliferation after transfection of our IR SSO or a non-specific control SSO with the Incucyte system. AAV8-eGFP transduction in OS and ES cells was performed alongside a GFP control plasmid over 72 hours in low-serum media.

Results: We observed a predominant IR-A:IR-B ratio in human OS and ES cell lines, dog OS PDXs, and human ES PDXs. To study this imbalance in IR alternative splicing, we transfected OS and ES cell lines with our 18-mer SSO targeting CUG-BP1 or a non-specific control 18-mer SSO at variable doses. We achieved at least a two-fold increase in IR-B expression in OS cell lines and a modest splicing switch in ES cell lines in a dose-dependent manner. This shift toward IR-B expression due to our SSO also significantly slowed OS cell proliferation as compared to the non-specific SSO. To address inefficient delivery in ES cells in vitro and OS tumor treatment in vivo, we designed various AAV plasmids to express the IR SSO sequence as an antisense RNA (aRNA) in transduced cells. Since splicing is a nuclear process, we used a promoter derived from the U7-snRNP particle, which has been well characterized in the literature to improve SSO entry into the nucleus and incorporation into the spliceosome to induce splicing changes. We have demonstrated successful transduction of AAV-8 eGFP vectors over a 72-hour time course in ES cells and are currently validating our aRNA-AAV candidate plasmids to identify the best candidate that switches IR splicing from IR-A toward IR-B.

Conclusion: Here, we confirm that OS and ES cells and PDXs preferentially express IR-A and that we can therapeutically modulate IR alternative splicing with our SSO in a dose-dependent manner. Additionally, our preliminary results indicate that AAVs, an FDA-approved methodology to deliver SSOs in a tissue-specific manner in pediatric diseases, are a promising

approach for pediatric sarcomas as well. Targeting IR alternative splicing with splice-switching therapies and AAV delivery has the potential to address a key resistance mechanism that rendered previously developed anti-IGF-IR therapies ineffective in pediatric sarcoma patients.

(P 95) LONG-TERM OUTCOMES OF PATIENTS DIAGNOSED WITH CHORDOMA OF THE SACRUM

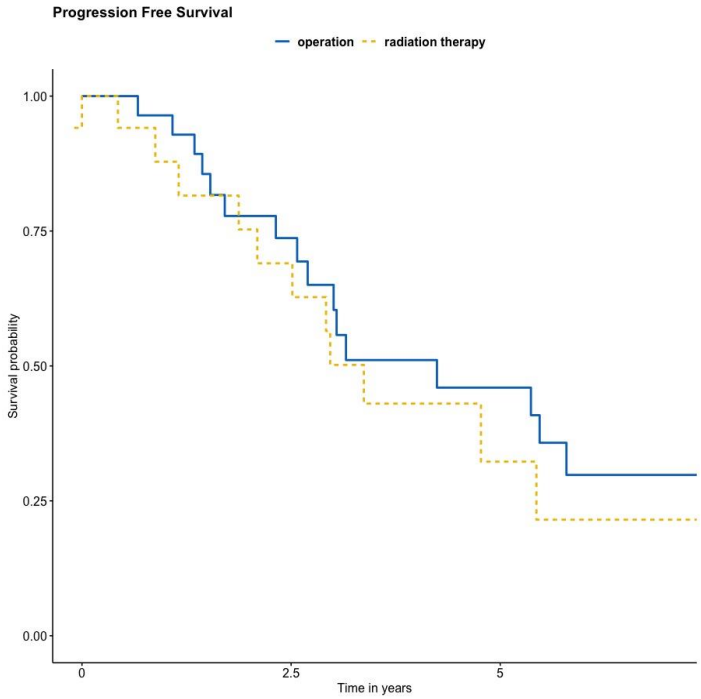
Aneta Borkowska, MD PhD[1]; Andrzej Pienkowski, MD, Phd[2]; Piotr L. Rutkowski, MD, PhD[3], (1) Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (2) Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (3) Maria Sklodowska-Curie National Research Institute of Oncology, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw Poland, Warsaw, Mazowieckie, Poland

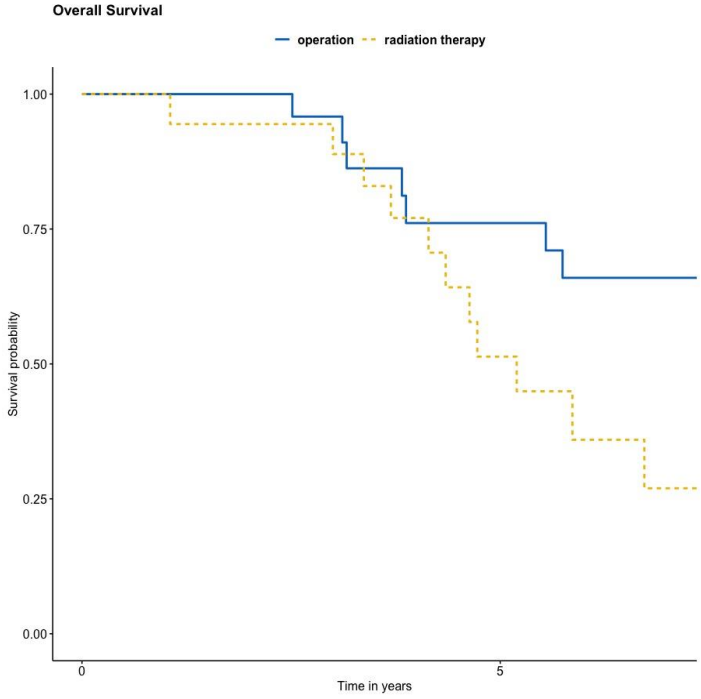
Objective: Oligoprogression is a limited progression after a prior response to treatment. Oligoprogression remains a major clinical challenge in patients with metastatic melanoma (MM). Local therapy for progressing metastases may enable the continuation of effective systemic therapy.

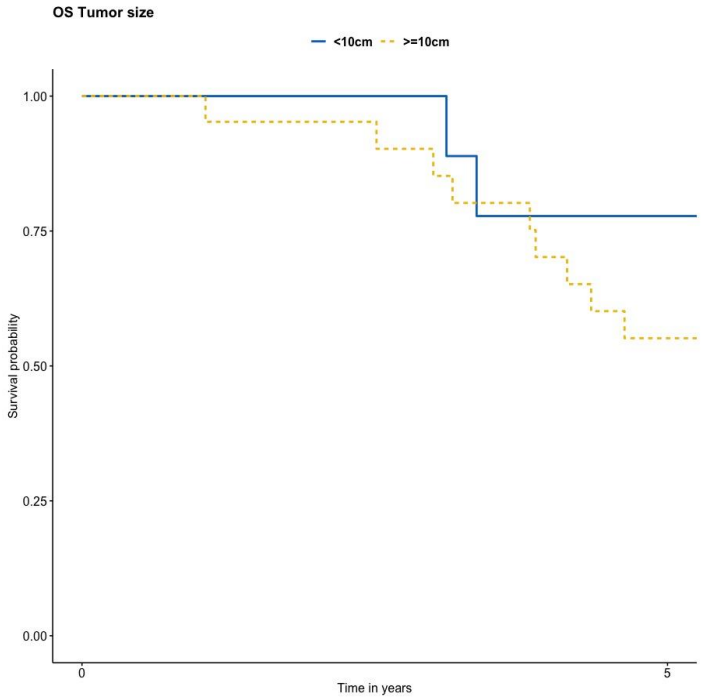
Methods: We identified 48 patients with chordoma of the sacrum diagnosis in a tertiary center between 2005 and 2020. We assessed patients' characteristics, progression free survival (PFS), and overall survival (OS) comparing surgery and RTH. The data cut-off was 10/Mar/2023.

Results: Majority of the group consists of men (58%). Twenty-nine of patients (60%) underwent initial surgery, with adjuvant RTH in 7 cases of inadequate resection. Rest of patients underwent RTH as primary treatment. The 3- and 5-year PFS rates in the whole group were 59% and 41%, respectively. The 3- and 5-year PFS rates were numerically better in the surgery group, 65% and 46%, respectively, compared to 3- and 5-year PSF results 50% and 32%, respectively in the RTH group (Fig. 1). Although, there was no statistical significance (p=0.59). The 3- and 5-year OS rates in the whole group were 92% and 65%, respectively. Survival rates in a group with initial surgery were significantly better compared to RTH (p=0.01, Fig. 2). The 3- and 5-year OS in the surgery group were 96%, and 76%, respectively, compared to the group of primary RTH with 89% 3-year OS, and 51% 5-year OS. The median size of lesions in the RTH group were bigger compared to the surgery group, 12cm versus 9cm, respectively. Nevertheless, there were no significant differences in survival (p=0.098, Fig. 3) nor local progression (p=0.5) between lesion size of < 10cm versus >=10cm. Interestingly, addiction of RTH in cases of recurrence after primary surgery did not improve survival outcomes (p=0.83). Also, the administration of salvage surgery after recurrence in the group of primary surgery was also not associated with improved survival outcomes (p=0.32).

Conclusion: En-bloc excision with broad margins stays the gold standard of sacral chordoma treatment. Radiotherapy can be useful in non-operative cases. The role of RTH as adjuvant after surgery with inadequate margins remains unclear.


Progression Free Survival
operation
radiation therapy
1.00
0.75
0.50
0.25
0.00
Survival probability
0
2.5
5
Time in years


Overall Survival
operation
radiation therapy
1.00
0.75
0.50
0.25
0.00
Survival probability
0
5
Time in years



(P 96) **LEPTOMENINGEAL RECURRENCE OF EWING'S SARCOMA: A CASE SERIES**

Fahad Faruqi, M.D., M.P.H[1]; Ayra Faruqi, MD[1]; Pedro Hermida De Viveiros, MD[2]; Jean-Paul Wolinsky, MD[2]; Seth Pollack, MD[3], (1) Northwestern Medicine, Warrenville, IL, (2) Northwestern Medicine, Chicago, IL, (3) Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL

Objective: Ewing sarcoma is a rare osseous malignant tumor that most commonly affects children and young adults. The tumor typically arises in the bones of the pelvis, limbs, or spine. Definitive treatment for localized disease often involves a combination of systemic therapy, radiation, and surgical resection. Leptomeningeal recurrence after definitive treatment of Ewing's sarcoma is a known but rare complication. Here we describe the clnical course and treatment outcomes of three patients found to have leptomeningeal disease after initial diagnosis of localized Ewing's Sarcoma

Methods: This was a retrospective case series of three patients with leptomeningeal recurrence of Ewing sarcoma. The patient's medical records were reviewed to obtain demographics, clinical history, pathology reports, imaging, treatment, and outcomes.

Results: Review of two females and one male, with a median age of 36 years. All patients had primary osseous involvement of Ewing sarcoma, with one patient having involvement of the zygoma arch, one patient having a sacral tumor, and one patient having a lumbar spine tumor. All three patients had evidence of extradural extension of the primary tumor. Pretreatment PET/CT imaging was performed in all reviewed cases and was negative for metastatic disease. None of the patients had any clinical evidence of focal neurologic deficits prior to initiation of definitive therapy. Two patients received initial neoadjuvant chemotherapy, followed by

definitive surgery or radiation therapy. The third patient received initial subtotal resection of her sacral tumor, followed by adjuvant chemotherapy and radiation therapy. All three patients eventually developed neurologic deficits with evidence of leptomeningeal disease within 6 months of completing definitive therapy for localized disease. All three patients received spinal proton radiation therapy, which resulted in significant improvement in symptoms. Post-radiation systemic therapy included gemcitabine in one patient and temozolomide/irinotecan in one patient.

Conclusion: This case series highlights the rare but devastating complication of leptomeningeal recurrence of Ewing sarcoma. All three patients in this study received salvage spinal proton radiation therapy, which resulted in significant improvement in symptoms. This study suggests that extradural extension of a primary Ewing's sarcoma spinal proton radiation therapy may be a promising treatment option for patients with leptomeningeal recurrence of Ewing sarcoma. This study was limited by its retrospective design. A prospective study with a larger sample size is needed to better understand the risk factors for leptomeningeal disease and the optimal treatment strategies. This study highlights the need for prospective studies to better understand the risk factors for leptomeningeal disease and to develop more effective treatment strategies. These studies could help to improve the outcomes for patients with this rare but devastating complication of Ewing sarcoma.

Immunology-Immunotherapy

(P 97) AN EXPLORATORY STUDY OF AN ANTI-PD-L1/TGF-β ANTIBODY, TQB2858, IN PATIENTS WITH REFRACTORY OR RECURRENT OSTEOSARCOMA AND ALVEOLAR SOFT PART SARCOMA: A REPORT FROM CHINESE SARCOMA STUDY GROUP

Lu Xie, MD[1]; Jie Xu, MD[1]; Xin sun, N/A, MD[1]; Xiaodong Tang, M.D.[1]; Xianan Li, M.D.[2]; Wei Guo, N/A, M.D.[1], (1) Peking University People's Hospital, Beijing, Beijing, China (People's Republic), (2) Hunan Cancer Hospital, Changsha, Hunan, China (People's Republic)

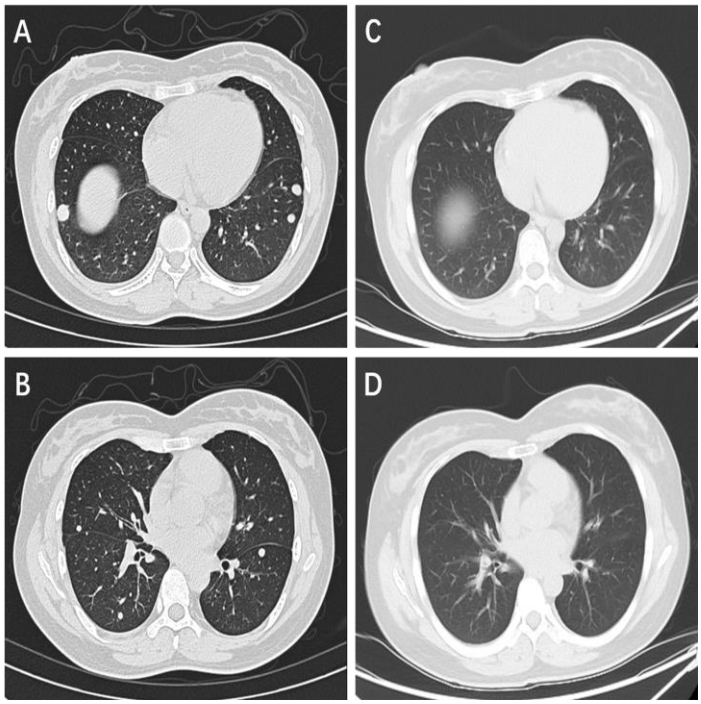
Objective: Novel effective immune-therapies are urgently needed in refractory or recurrent sarcoma. Transforming Growth Factor-Beta (TGF-β) is a diverse regulatory and fibrogenic protein expressed in multiple sarcoma tumors, which promotes epithelial-mesenchymal transition and excessive deposition of extracellular matrix. This study evaluated efficacy and safety of anti-PD-L1/TGF-β antibody TQB2858 in patients with refractory osteosarcoma or alveolar soft part sarcoma (ASPS).

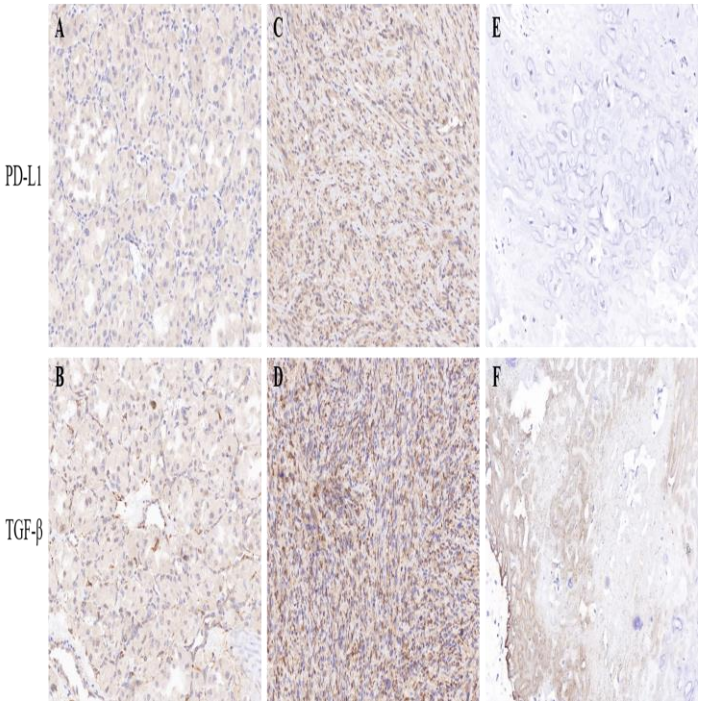
Methods: In this single-arm Phase 1b exploratory study, we included patients with refractory osteosarcoma with progressive disease who had received at least 2 lines of systemic therapy previously or ASPS at least one line of systemic therapy. Patients were administered with 1200mg TQB2858 on day 1 every 3 weeks. The primary endpoint was Objective Response Rate (ORR), with null and alternative hypotheses of ORR≤5% and ≥20%, respectively. Exploratory biomarker analyses with immunohistochemistry (IHC) staining (PD-L1 and TGF-β) were performed on pre-treatment tumor samples.

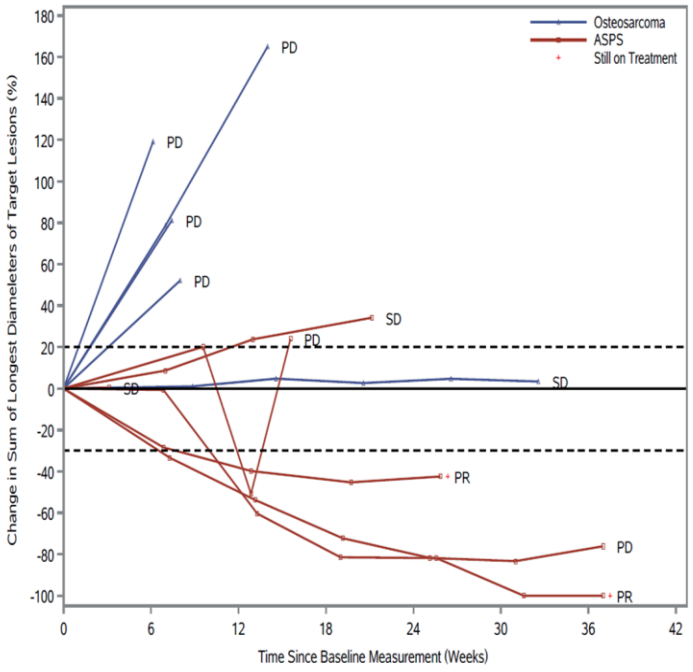
Results: Eleven eligible patients were included in the outcome analysis. TQB2858 did not

demonstrate evidence of efficacy as 0/5 osteosarcoma had any objective response while 2/6 ASPS showed partial response, of whom the median progression free survival were respectively 1.51 (1.38, NE) and 2.86 (1.38, NE) months for osteosarcoma group and ASPS group. None of the administered therapy cycles met criteria for unacceptable toxicity. Other Grade 3 toxicities included abnormal liver function and elevation of γ-glutamyltransferase. IHC analysis revealed that functional enrichment neither in the TGF-β pathway or in PD-L1 was not associated with treatment outcome.

Conclusion: The combination of PD-L1 and TGF-β antibody TQB2858 did not significantly improve ORR in recurrent osteosarcoma. However, it did improve the immunogenic responses in ASPS even after progression upon anti-PD-1/PD-L1 therapy with an acceptable safety profile. IHC profiling with pathway enrichment analysis would have possibilities to have predictive value for survival outcome, necessitating further investigation to confirm.




A
C
E
PD-L1
B
D
F
TGF-β


Osteosarcoma
ASPS
Still on Treatment
Change in Sum of Longest Diameleters of Target Lesions (%)
Time Since Baseline Measurement (Weeks)
PD
PD
PD
PD
SD
PD
SD
SD
PR
PD
PR
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
6
12
18
24
30
36
42

(P 98) TUMOR IMMUNE PROFILING AND IDENTIFICATION OF IMMUNOTHERAPEUTIC TARGETS IN DEDIFFERENTIATED LIPOSARCOMA

Anna Jirovec, BSc[1]; Ashley Flaman, MD[2]; Bibianna Purgina, MD FRCPC[2]; Andrew Chen, BSc[1]; Joel Werier, FRCSC[2]; Jean-Simon Diallo, PhD[3], (1) Ottawa Hospital Research Institute, Ottawa, ON, Canada, (2) The Ottawa Hospital, Ottawa, ON, Canada, (3) The Ottawa Hospital Research Institute, Ottawa, ON, Canada

Objective: The poor prognosis of patients with soft-tissue sarcoma has not changed in the past several decades, highlighting the necessity for new therapeutic approaches. T-cell based immunotherapies are a promising alternative to traditional cancer treatments due to their ability to target only malignant cells, leaving benign cells unharmed. The development of successful immunotherapy requires the identification and characterization of targetable immunogenic tumor antigens. Cancer-testis antigens (CTA) are a group of highly immunogenic tumor-associated proteins that have emerged as potential targets for CD8+ T-cell recognition. In addition to identifying a targetable antigen, it is crucial to understand the tumor immune microenvironment. The level of immune infiltration and mechanisms of immune suppression within the tumor play important roles in the outcome of immunotherapy. The goal of this study is to identify targetable immunogenic antigens for T-cell based immunotherapy and to characterize the tumor immune microenvironment in human dedifferentiated liposarcoma (DDLS) by Nanostring and IHC. In addition, a prime-boost vaccination employing Modified Vaccinia virus Ankara (MVA) and the oncolytic Vesicular stomatitis virus (VSV) targeting a CTA is evaluated in a murine model of sarcoma.

Methods: To assess the complexity of the human DDLS tumor immune microenvironment and to identify target antigens we used the nCounter NanoString platform to generate a gene expression profile for hundreds of genes from RNA obtained from 29 DDLS and 10 control fat FFPE samples. To classify inflammatory status of DDLS tumors, we performed hierarchical clustering based on expression levels of selected tumor inflammatory signature genes (CCL5, CD27, CD274, CD276, CD8A, CMKLR1, CXCL9, CXCR6, HLA-DQA1, HLA-E, IDO1, LAG3, PDCDILG2, PSMB10, STAT1, TIGIT). To confirm protein expression and distribution of identified antigens, we performed immunohistochemistry on human tissue micro-arrays encompassing DDLPS tumor tissues and matched normal control tissue from 63 patients. IHC for the cancer testis antigens PBK, SPA17, MAGE-A3, NY-ESO-1 and SSX2 was performed, and positive staining was determined by two authors. The top candidate antigen was cloned into the MVA prime and VSVΔ51 boost vectors. Naïve BALB/c mice were immunized with 107 PFU MVA-PBK (i.m.) prime at day 0 and 108 PFU of VSVΔ51-PBK (i.v.) boost at day 14. The antigen specific immune response was assessed by IFNγ ELISPOT assay and by intracellular cytokine staining (ICS). Next, naïve BALB/c mice were immunized with prime and boost vaccinations, and challenged 7 days post-boost by subcutaneous injection with either fibrosarcoma (WEHI-164) or K7M2 (osteosarcoma) murine sarcoma cell lines expressing PBK, tumor growth was monitored for 30 days.

Results: Hierarchical clustering of DDLS tumors based on expression of tumor inflammation signature genes revealed two distinct groups, consisting of 15 inflamed tumor and 14 non-inflamed tumors, demonstrating tumor heterogeneity within the DDLS sarcoma subtype. Notably, antigens PBK and TTK had the largest fold change increase in expression in DDLS compared to normal fat controls. Immunohistochemical analysis of selected antigens revealed that PBK was expressed in 96% (52/54) of DDLS samples at high levels. Other antigens were absent or expressed at low levels in DDLS; MAGEA3 in 15.87% (10/63) NY-ESO-1 in 6.35% (4/62) and SSX2 in 12.7% (8/63) and SPA17 in 5.5% (3/54). Immunization with prime and boost

vectors generates a strong cellular antigen specific response compared to controls. Prophylactic immunization of mice with MVA-PBK and VSVΔ51-PBK significantly reduced K7M2 tumor growth and compared to PBS controls.

Conclusion: This data shows considerable inter-tumoral heterogeneity of inflammation, which should be taken into consideration when designing an immunotherapy for DDLS. To date, these results show promising expression of PBK antigen in DDLS, which may be used as a target in the future development of an immunotherapy for sarcoma.

(P 99) M2 MACROPHAGE INFILTRATION IS ASSOCIATED WITH EARLY DISEASE RECURRENCE FOLLOWING CURATIVE INTENT SURGICAL RESECTION IN SOFT-TISSUE SARCOMAS

Nathan D. Seligson, PharmD[1]; Yan W. Assmann, PhD[2]; Tariq Almerey, MD[3]; Yaquelin Coll Zayas, BS[1]; Steven Attia, D.O.[2]; Keith Knutson, PhD[3]; Sanjay Bagaria, MD[2], (1) University of Florida, Jacksonville, FL, (2) Mayo Clinic, Jacksonville, FL, (3) The Mayo Clinic, Jacksonville, FL

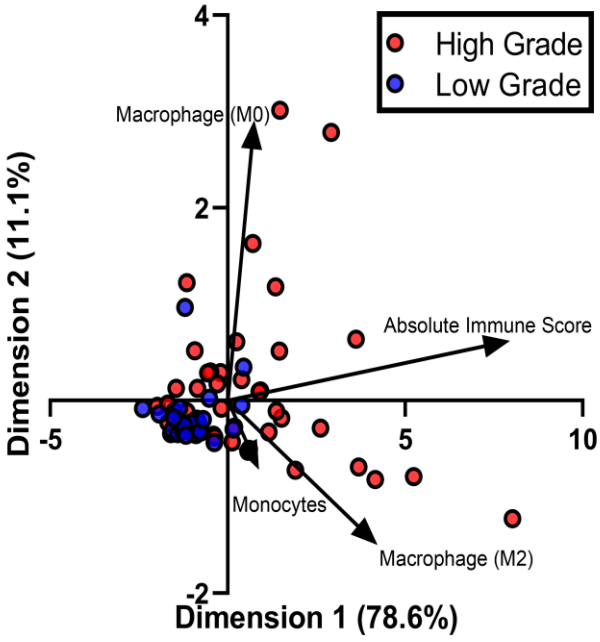
Objective: The clinical management of soft-tissue sarcomas (STS) is challenging with aggressive surgical resection offering the only chance for cure. Unfortunately, a large proportion of patients develop local recurrence and distant metastasis. Despite advances in therapy for STS, there are no clear clinical guidelines on when or what local or systemic therapy to use in combination with surgical resection or which subsets of patients would benefit from these additional interventions. There is a critical need to improve the diagnosis, prognosis, and treatment of early stage STS using molecular diagnostic and precision medicine approaches. The tumor-immune microenvironment (TIME) represents a vital component of cancer and is an emerging prognostic and predictive biomarker across many malignancies. In STS, the TIME is thought to be cold relative to other cancers; however, recent evidence suggests that subgroups of STS demonstrate a more active TIME. In this study we estimated immune cell infiltration using CIBERSORTx in 69 subjects with early stage STS who received curative intent surgical resection to determine the prognostic value of TIME and identify preliminary biomarkers for targeted therapy.

Methods: Primary, retroperitoneal, formalin-fixed, paraffin-embedded (FFPE) tumor samples were selected from the Mayo Clinic Florida STS Biobank and sequenced using the Tempus xT targeted DNA and whole transcriptome next-generation sequencing platforms. Immune cell deconvolution was calculated using the original CIBERSORT method within the CIBERSORTx deconvolution algorithm. B mode batch correction was applied and the LM22 signature was used as the immune reference. CIBERSORTx was run using both relative and absolute modes with quantile normalization disabled and the deconvolution was estimated for 1,000 permutations. Clinical endpoints included overall survival (OS) and disease-free survival (DFS). OS was defined as time from initial surgical resection to date of death or last known follow-up. DFS was defined as time from initial surgical resection to date of first local recurrence, first metastasis, or last known follow-up. Tumor grade was defined as high (grade 3) or low (grades 1-2). Principle component analysis (PCA) was conducted using the FactoMineRv2.3 package in R. Survival analysis was tested using both log-rank and Cox proportional hazard methods. Unless otherwise stated, p-values ≤0.05 were considered statistically significant.

Results: Of the 69 included STS, the most common histologies included were liposarcoma

(n=22), gastrointestinal stromal tumor (n=19), and leiomyosarcoma (n=12). Immune cell infiltration was highly variable across the cohort. Immune infiltration was not associated with tumor purity (p=0.56). The most abundant cell types identified included M2 macrophages, resting T-cells, monocytes, and resting mast cells. Immune infiltration was similar across tissue histologies; however, GIST demonstrated consistently low immune infiltration. Low-grade tumors also demonstrated a lower Immune infiltration compared to high-grade tumors. Using principle component analysis (PCA), we determined that immune infiltrate heterogeneity was primarily driven by the prevalence of absolute immune cells, macrophages (M0 and M2), and monocytes (Figure). Unsurprisingly, M2 macrophages were the highest correlated cell type with absolute immune scores and were the dominant cell type identified across the dataset. While M0 macrophages were not highly prevalent in this cohort, their impact on tumor clustering is likely due to their inverse relationship with M2 macrophages. To determine the correlation between immune cell infiltration and clinical outcomes in this dataset, we took a two-step approach, requiring gene sets to be correlated with both DFS and OS first as categorical variables (median split, $p< 0.05$), then as continuous variables (z-scores, $p< 0.1$). Only M2 macrophage infiltration was associated with clinical outcomes with high infiltration associated with poor DFS and OS.

Conclusion: Prior studies have associated STS with cold immune environments; however, with the high infiltration present in a small number of tumors in this dataset, it is possible that there may still be a spectrum of immune environments in STS. We identified a M2 macrophage dominant, pro-tumor TIME in high-risk disease. Taken together, this data may help to identify patients receiving curative intent surgical resections for intra-abdominal STS who are at the highest risk of recurrence, while presenting the potential target for immunotherapy in a subset of high-risk STS.



(P 100) HISTOLOGY SPECIFIC INTRATUMORAL B-CELL BIOLOGY IN ALVEOLAR SOFT-PART SARCOMAS (ASPS) IN RESPONSE TO DURVALUMAB-TREMELIMUMAB (D-T)

Elise F. Nassif, MD[1]; Hannah Beird, PhD[2]; Akash Mitra, PhD[3]; Bin Liu, PhD[4]; Kevin McBride, PhD[4]; Wei-Lien Wang, M.D[5]; Davis R. Ingram, BS[5]; Khalida Wani, PhD[4]; Emily Z. Keung, MD, AM, FACS[5]; Rare Tumor Initiative Team Patient Mosaic Team, N/A[4]; Maria A. Zarzour, MD[4]; Anthony P. Conley, MD[4]; Dejka M. Araujo, MD[5]; Andy Livingston, MD, MS[5]; Ravin Ratan, MD[5]; Shreyaskumar Patel, MD[5]; Vinod Ravi, MD, MS, MBA[5]; Robert Benjamin, MD[5]; Alexander J. Lazar, MD, PhD[5]; Christina Roland, MD, MS[5]; P Andrew Futreal, PhD[5]; Neeta Somaiah, MD[5], (1) University of Texas MD Anderson Cancer Center, Houston, TX, (2) Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA, Houston, TX, (3) Tempus Labs, Houston, TX, (4) MD Anderson Cancer Center, Houston, TX, (5) The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: ASPS is characterized by the ASPSCR1-TFE3 translocation and exceptional response to immunotherapy as demonstrated in the recent phase II clinical trial in advanced sarcoma patients with select histotypes, including those with ASPS. In other tumor types (undifferentiated pleomorphic sarcomas [UPS], melanoma, renal cell carcinoma), intratumoral B-cells and tertiary lymphoid structures (TLS) are important predictive biomarkers of response to immunotherapy. Therefore, we sought to investigate the intratumoral B-cell biology of ASPS patients compared to other patients included in this trial.

Methods: This trial enrolled 62 sarcoma patients (10 ASPS, 6 liposarcoma, 5 angiosarcoma, 5 leiomyosarcoma, 5 UPS, 5 synovial sarcoma, 5 osteosarcoma, 5 chordomas, 11 other). The primary endpoint of this trial was progression-free rate at 12 weeks. Longitudinal biospecimen collection included biopsies at baseline (n=56; ASPS n=8), at week 6 (optional; n=43; ASPS n=8), and at progression (optional, n=2). Whole hematoxylin and eosin slides were evaluated for presence of lymphoid aggregates (LA) by an experienced sarcoma pathologist, which was used as a surrogate marker for TLS and reported as present or absent. Biopsies were stained for CD20 by immunohistochemistry (IHC). The number of CD20 positive cells quantified and reported as cell densities. RNA sequencing (RNAseq) was performed and immune cell populations were inferred using MCPcounter immune deconvolution method and reported as z-scores. Intratumoral B-cell receptor (BCR) repertoire at baseline was evaluated by bulk tumor RNAseq using the TRUST4 algorithm and BCR repertoire diversity and clonal distribution were assessed by inverse Simpson index and Gini coefficient, respectively. Comparisons of continuous variables were performed using Wilcoxon Rank-Sum test and categorical variables using Fisher's exact test.

Results: ASPS samples showed no increase in the proportion of samples with presence of LA ($p > 0.9$) whereas non-ASPS samples showed a significant increase in the number of samples with presence of LA with D-T ($p = 0.038$; Figure 1A). ASPS samples showed a non-significant decrease in CD20 densities with D-T ($p = 0.22$), whereas non-ASPS samples had an increase in CD20 positive cell densities with D-T ($p = 0.012$; Figure 1B). At baseline, ASPS had higher densities of CD20 positive cells compared to non-ASPS ($p = 0.025$; Figure 2A) which was confirmed with RNAseq ($p < 0.001$; Figure 2B). Concerning the BCR repertoire, there were no significant differences in diversity between ASPS and non-ASPS patients, neither for the heavy nor light chains. However, there was a non-significant trend for greater clonality in ASPS compared to non-ASPS both in the heavy and light chains at baseline and after D-T (Figure 3).

Conclusion: The way in which the B-cells in ASPS may contribute to the exceptional

responses seen with D-T may be related more to the pre-existing numbers of B-cells and independent of lymphoid aggregation. Further work in this area should focus on the mechanisms underlying this ASPS-specific intratumoral B-cell and to enhance presence of B-cells in other sarcoma types. Additional analyses on these trial samples are ongoing to further delineate the role B-cells in ASPS response to D-T.

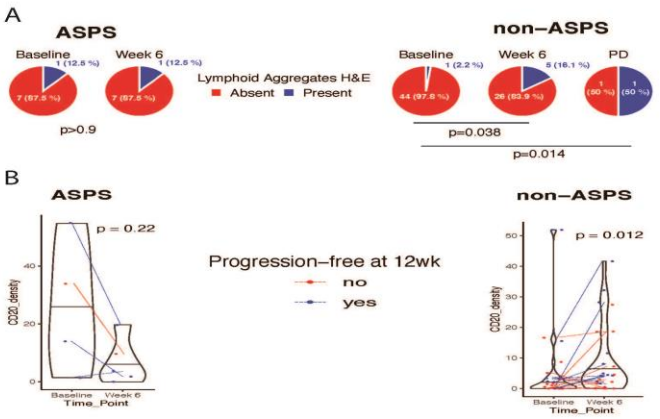


Figure 1: Intratumoral B-cell and Lymphoid Aggregates Dynamics with Durvalumab-Tremelimumab in ASPS and non-ASPS
A. Lymphoid Aggregates
B. B-cells by CD20 IHC density

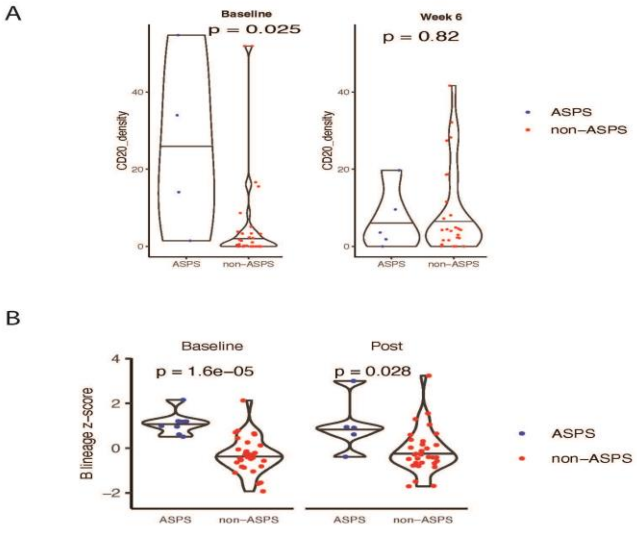


Figure 2: ASPS have Higher Intratumoral B-cell Density Than non-ASPS
A. By CD20 IHC density
B. By immune deconvolution of RNA sequencing

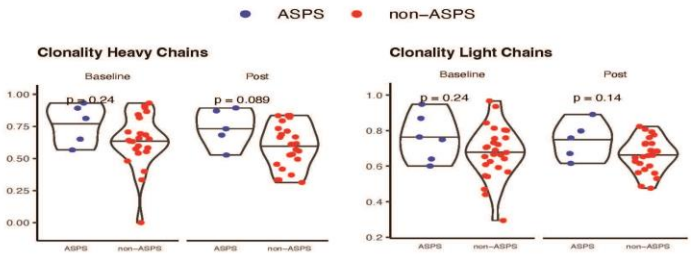


Figure 3: ASPS have Higher Intratumoral B-cell Receptor Repertoire Clonality

(P 101) EPIGENETIC MODULATION OF NK CELLS IMPROVES TUMOR TRAFFICKING AND ENHANCES THERAPEUTIC EFFICACY AGAINST OSTEOSARCOMA

Ariana R. Anjier, MS, PhD[1]; Pradeep Shrestha, PhD[2]; Shinji Maegawa, PhD[1]; Vidya Gopalakrishnan, PhD[3]; Juan Bournat, PhD[2]; Donghang Cheng, PhD[2]; Yanwen Yang, PhD[2]; Charles V. Kingsley, n/a[2]; Adam Kulp, n/a[2]; Eugenie S. Kleinerman, MD[1]; Nancy Gordon, MD[3], (1) University of Texas MD Anderson, Houston, TX, (2) MD Anderson Cancer Center, Houston, TX, (3) The University of Texas MD Anderson Cancer Center, Houston, TX

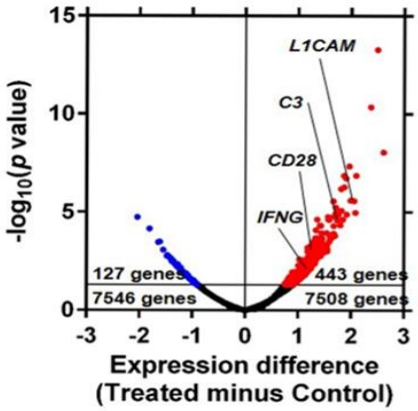
Objective: Osteosarcoma (OS) remains a difficult disease to treat with 30-35% of patients dying of pulmonary metastases. Immunotherapy is becoming the main stay treatment in cancer. However, immune therapy and checkpoint inhibitors for relapsed OS has shown limited or unsatisfactory results. Natural Killer (NK) cells have demonstrated anti-tumor activity against OS. However, therapeutic efficacy has been limited due to the inability to penetrate, proliferate, and survive within the tumor. Therefore, strategies to improve NK cell efficacy are needed. We showed that the histone deacetylase inhibitor (HDACI) MS-275, immune-sensitized OS cells to NK cell-mediated lysis. MS-275 has also been shown to augment NK cell function. Therefore, the objective of our studies was to determine if epigenetic modulation of allogeneic NK cells with the HDAC inhibitor MS-275 could improve tumor trafficking and cytolytic function, thus enhancing therapeutic efficacy against OS lung metastases.

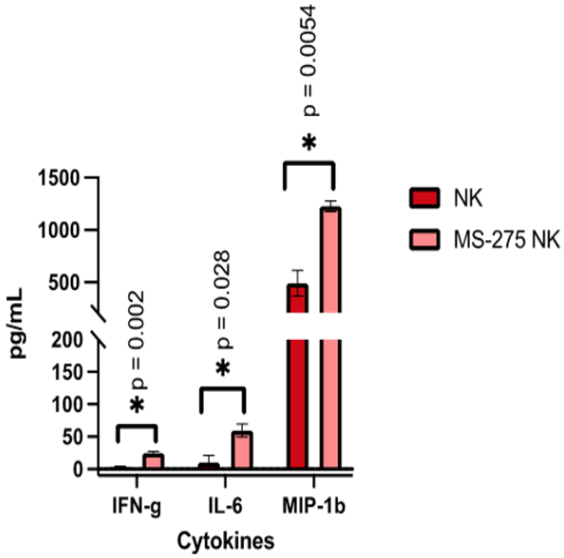
Methods: Human NK cells were purified and expanded from donor buffy coats using recombinant human IL-2 and genetically engineered K562 feeder cells. RNA was extracted from untreated and MS-275 pre-treated NK cells, submitted for RNA seq and ChiPseq. Additionally, culture media was collected for cytokine analysis. NK cells were also analyzed by flow cytometry to measure differences in CD56, CD16, NK activating receptor NKG2D, Ki67, Granzyme B and IFN**γ**.
NSG mice were injected with human OS17 luciferase-labeled OS cells and divided into three groups (untreated, NK cell treatment, and MS-275 pre-treated NK cell treatment) when lung

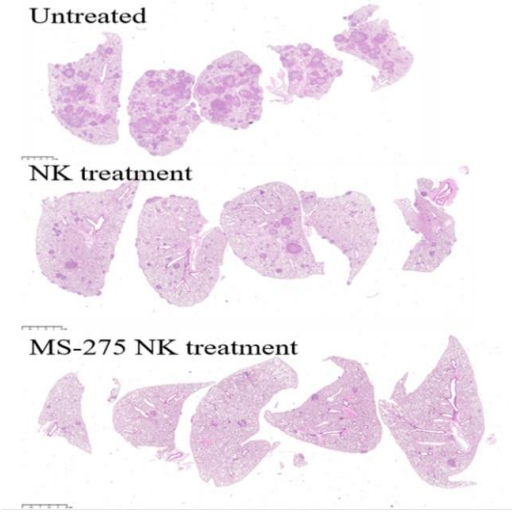
tumors were confirmed by bioluminescence. NK cells were treated for 24 hours with 0.5uM MS-275 prior to IV injection into mice and administered twice/week for at least two weeks with some NK doses stained with near-infrared DilC18: 1.1”-dioctadecyl-3,3,3’,3’-tetramethylindotricarbocyanide iodide (DiR) fluorescent dye prior to injection to allow for tracking of NK cells in-vivo using the IVIS imaging system. Mice were either euthanized 24 hours after the final NK cell treatment for histologic analysis of the lungs or, analyzed for survival across treatment groups. Additional mice from each group were used for 3D imaging [Xerra Cryofluorescent tomography (CFT)].

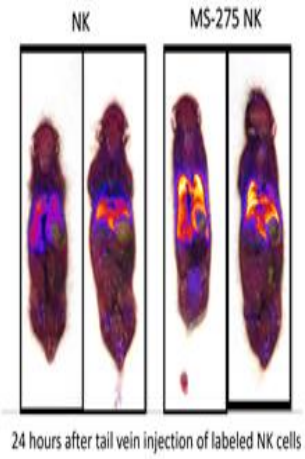
Results: MS-275 upregulates expression of immunomodulatory genes on NK cells. 547 differentially expressed genes were identified between the untreated and MS-275 treated NK cells. Four immune related genes (L1CAM, IFNG, C3 and CD28) were upregulated in the MS-275 treated NK cells and histone H3 and H4 acetylation was found at the identified immunomodulatory genes. Culture media from MS-275 treated NK cells showed significantly increased MIP-1b, IL-6 and IFN-g compared to untreated NK cells. MS-275 treated NK cells have increased Ki67, Granzyme B and IFN**γ**, measured by flow cytometry, indicating increase proliferation and cytotoxic function.
MS-275 pre-treatment enhanced therapeutic efficacy of NK cells against OS. There was a significant decrease in micro-metastases in the MS-275 pre-treated NK cell group as compared to the untreated control group (199 vs 9 p=0.05). Lastly, Xerra imaging demonstrated greater DiR signal in the lungs of the mice that received MS-275 treated NK cells compared to those that received untreated NK cells, suggesting greater persistence of NK cells at tumor sites.

Conclusion: We conclude that MS-275 has an immunomodulatory effect on NK cells (increased L1CAM, IFN**γ**, C3 and CD28) and increases H3 and H4 acetylation suggesting transcriptional activation of the immune-related genes that contribute to NK cell increased cytolytic function. The increase in Ki67, Granzyme B and IFN**γ** seen with treatment of NK cells with MS-275 suggests that treated cells have increased proliferation and cytotoxic function. Pre-treatment of NK cells with MS-275 enhances NK cell therapeutic efficacy by enhancing localization and persistence within the lungs.









24 hours after tail vein injection of labeled NK cells

(P 102) INTERLEUKIN-6 IN RELATION TO TUMOR BEHAVIOR AND EARLY RECURRENCE IN PRIMARY LOCALIZED SOFT TISSUE SARCOMA

Pia van der Laan, MD[1]; Winette van der Graaf, Prof. Dr.[2]; Daan van den Broek, Dr.[1]; Hester H. van Boven, Dr.[1]; Neeltje Steeghs, Dr.[2]; Winan J. van Houdt, MD, PhD, MS[2], (1) Netherlands Cancer Institute/Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (2) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands

Objective: The diversity of clinical and pathological characteristics of soft tissue sarcoma (STS) presents a challenge for the prediction of prognosis. Recently, pretreatment inflammatory markers have gained interest as predictive factors for tumor aggressiveness and survival in cancer patients. The aim of this study is to evaluate interleukin-6 (IL-6) as a predictive biomarker for aggressive behavior and early recurrences in STS.

Methods: 118 patients with primary, localized soft tissue sarcoma were retrospectively reviewed. IL-6 serum levels were obtained prior to treatment for all patients. Early recurrence was defined as a disease relapse (local or distant) within the first year after diagnosis. The association of pretreatment elevated IL-6 was studied in relation to clinicopathological variables including tumor grade, tumor depth, tumor location, tumor size, presence of tumor necrosis on imaging, pathologic necrosis and mitotic count by using Chi-square tests. Survival data was analyzed using Log-rank tests and Cox regression to identify prognostic factors for early recurrence.

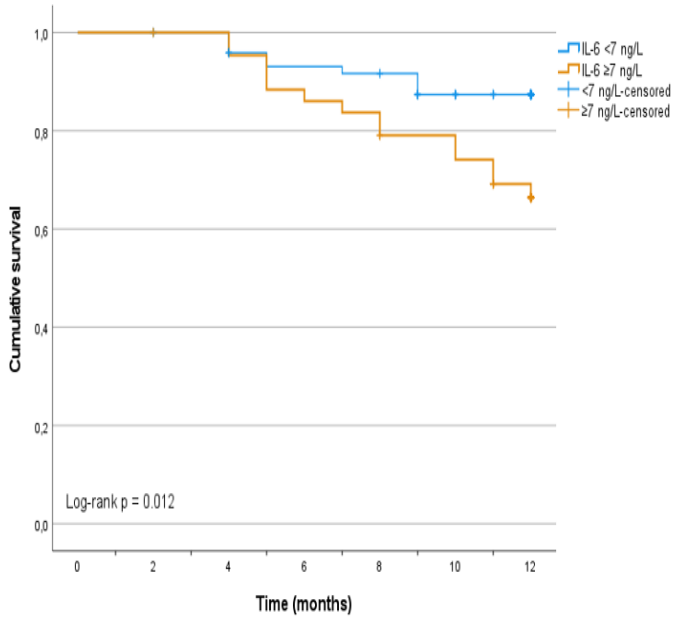
Results: Pretreatment IL-6 elevation was associated with higher tumor grade, increased size, necrosis on imaging, pathologic necrosis and a higher mitotic count. Tumor depth and location were not associated with IL-6 level. Early recurrences were found in 23 of 118 patients (19.5%): 7 local, 15 distant recurrences and 1 with both manifestations. Univariate analysis revealed that tumor grade (low vs high, $p = 0.008$), tumor depth (superficial vs deep, $p = 0.005$), pathologic necrosis (absent vs present, $p = 0.002$) and IL-6 serum level (< 7 vs ≥ 7 ng/L, $p = 0.012$) were significantly associated with early recurrence. IL-6 level did not retain its prognostic value in multivariate analysis as independent predictor for early recurrence, although the number of cases were small.

Conclusion: Increased IL-6 serum level in STS is associated with an aggressive phenotype. STS that recur early are more often of higher grade and deep-seated, but are also associated with increased plasma levels of IL-6.

Table 1. Relation of pretreatment IL-6 level and clinicopathological features. HPF: high power field.

Variable, n (%)	IL-6 level <7 ng/L	IL-6 level ≥7 ng/L	P-value
Grade			<0.001
1	23 (35.9)	1 (2.4)	
2-3	41 (64.1)	41 (97.6)	
Size			<0.001
< 5 cm	25 (33.8)	5 (11.4)	
5 -10 cm	32 (43.2)	12 (27.3)	
> 10 cm	17 (23.0)	27 (61.4)	
Location			0,163
Retroperitoneal, intra-abdominal, pelvic	18 (24.3)	16 (36.4)	
Other	56 (75.7)	28 (63.6)	
Depth			0,124
Superficial	21 (28.4)	7 (15.9)	
Deep	53 (71.6)	37 (84.1)	
Necrosis on imaging			0.001
Absent	64 (87.7)	26 (61.9)	
Present	9 (12.3)	16 (38.1)	
Pathologic necrosis			0.001
Absent	33 (58.9)	10 (25.7)	
Present	23 (41.1)	29 (74.4)	
Mitotic count			0.026
0-9 mitoses/10 HPF	41 (68.3)	16 (41)	
10-19 mitoses/10HPF	9 (15)	12 (30.8)	
≥ 20 mitoses/10HPF	10 (16.7)	11 (28.2)	

Figure 1. Kaplan Meier for early disease recurrence according to IL-6 groups



(P 103) LASTING COMPLETE RESPONSE TO PEMBROLIZUMAB IN MISMATCH REPAIR DEFICIENT CARDIAC SARCOMA: A GENOMIC CHARACTERIZATION

Daniela A. Ferraro, MD/PhD[1]; Bettina Bisig, MD[1]; Fresia Pareja Zea, MD PhD[2]; David C. Rotzinger, MD, PhD[1]; Edoardo Missiaglia, MD[1]; Krisztian Homicsko, MD PhD[1]; Antonia Digklia, Dr Med[3], (1) CHUV, Lausanne, Vaud, Switzerland, (2) MSKCC, New York, NY, (3) Department of Oncology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland., Lausanne, Vaud, Switzerland

Objective: Tumor-infiltrating lymphocytes (TILs), tumor mutation burden (TMB), and PD-L1 expression are the most important predictive markers of response to immune check-point inhibitors (ICIs) in epithelial tumors. Sarcomas are traditionally considered “cold” tumors with poor response to immunotherapy. However, the genetic landscape of sarcoma is markedly heterogeneous, and rare sarcoma cases harbor a high TMB and/or high numbers of TILs. We present the case of a 73-year-old woman with a mismatch repair deficient (MMRd) undifferentiated cardiac sarcoma, who achieved a complete and durable response with the anti-PD1 inhibitor pembrolizumab after the failure of standard treatment consisting of doxorubicin together with olaratumab.

Methods: The tumor resection specimen was routinely processed to establish the histopathologic diagnosis. In addition, MMR proteins (MLH1, PMS2, MSH2, MSH6), PD-L1 (clone SP263) and CD8+ T cells were assessed by immunohistochemistry (IHC). MLH1 gene promoter methylation was evaluated by pyrosequencing. High-throughput sequencing (HTS) was performed on tumor DNA and matched constitutional DNA, using a customized 400-gene panel (full coding sequences, covering a total of 1.24 Mb). TMB and mutational signature were determined based on somatic variants.

Results: A 73-year-old woman presented in September 2016 progressive dyspnea. Magnetic resonance imaging (MRI) revealed a left atrium tumor, which was diagnosed as primary cardiac undifferentiated sarcoma (UDS), in the absence of MDM2 gene amplification. After a first line therapy with doxorubicine and olaratumab, progressive disease developed in May 2018, prompting us to analyze predictive markers in the initial resection specimen. Tumor cells showed a loss of MLH1 and PMS2 expression (with preserved MSH2 and MSH6). They were negative for PD-L1 (tumor proportion score (TPS) < 1%), while a subset of tumor-associated immune cells was positive (combined positive score (CPS) >20). TILs were abundant, with a mean of >20 intratumor CD8+ T cells per high power field (400x magnification). HTS revealed numerous somatic variants (Table 1), accounting for a TMB of 12.9 non-synonymous somatic mutations per Mb. Variants were mostly transitions and small indels in homopolymeric sequences, consistent with an MSI-associated mutational signature. Interestingly, a somatic splice variant was detected at the intron 1 / exon 2 boundary of MLH1 (c.117-3C>G), and was associated with a copy gain of the mutated allele and a loss of heterozygosity (loss of the wild-type allele). In the absence of MLH1 promoter hypermethylation (as determined by pyrosequencing), we attributed the loss of expression of MLH1 by IHC to this splice variant. Other relevant findings of HTS included a missense mutation in TP53, and frameshift deletions in FAT1 and NOTCH2 (Table 1).
Based on these results, the patient started treatment with the anti-PD-L1 antibody pembrolizumab, in May 2018 for 14 cycles in total, and was then discontinued due to side effects. The disease's follow-up by MRI showed that partial response started 6 months after the beginning of pembrolizumab, and complete response was achieved several months after discontinuation and continues to persist at the last MRI in March 2022. (Figure 1)

Conclusion: The genomic analysis of the cardiac UDS displayed several markers known to predict response to ICIs: the tumor harbored a high TMB (> 10 mutations/Mb) and a MMRd/MSI signature. Of note, inactivating mutations of both FAT1 and NOTCH2 have also been associated with ICI therapy response in epithelial tumors. Moreover, the tumor displayed an extensive infiltrate by CD8+ T cells, and PD-L1 was expressed in the tumor immune microenvironment. Based on these biomarkers and given the paucity of other treatment options acceptable to the patient, she was treated with pembrolizumab monotherapy and obtained a durable response.

Although immunotherapy may have a role in some sarcoma patients, the identification of suitable candidates who can obtain a meaningful benefit from treatment requires improvement. Considering the genomic variability between sarcomas and the heterogeneous response to ICIs, validation of new predictive markers is essential to improve the efficacy of immunotherapy in sarcomas. The use of a combination of existing biomarkers may improve their predictive power. This might allow for a more personalized approach and an increase response rate to immunotherapy in diseases that are still considered resistant.

Figure 1

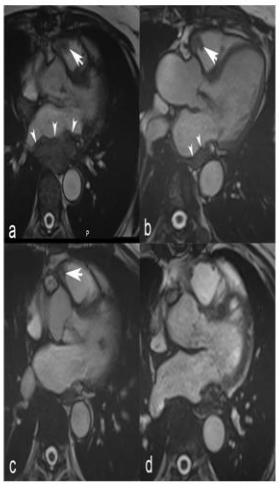


Fig. 1 legend: steady-state free precession cardiac MRI scans show masses in the right ventricular outflow tract (white arrow) and left atrium (white arrowheads) at baseline (**panel a**).Six months later the beginning of treatment with pembrolizumab (**panel b**), a follow-up MRI showed a slight decrease of the mass located in the right ventricular outflow tract (white arrow) and notable shrinkage of the left atrial mass that is merely visible as an atrial wall thickening (white arrowheads). Further tumor shrinkage of the right ventricular outflow tract mass (white arrow) was observed after discontinuation of treatment for adverse events (**panel c**), and ultimately complete response with no residual tumor at the last control (**panel d**).

Table 1. Somatic mutations detected by high-throughput sequencing (400-gene panel).

Gene	Exon	Reference transcript	Mutations	Variant allele frequency	Coverage	Pathogenicity class§
TP53	7	NM_000546.5	c.742C>T, p.(Arg248Trp)	46%	241X	5
FAT1	10	NM_005245.3	c.8799del, p.(Gly2934Valfs*3)	33%	878X	4
NOTCH2	34	NM_024408.3	c.6650_6651del, p.(Val2217Alafs*26)	17%	846X	4
MLH1	2	NM_000249.3	c.117-3C>G (splice site)	69%	464X	4
BAP1	6	NM_004656.3	c.436A>T, p.(Arg146Trp)	53%	481X	3
CBFB	1	NM_022845.2	c.54del, p.(Phe18Leufs*4)	29%	519X	3
PTPRT	3	NM_133170.3	c.444G>T, p.(Lys148Asn)	21%	840X	3
PNRC1	1	NM_006813.2	c.332C>A, p.(Pro111His)	20%	2290X	3
LRP6	6	NM_002336.2	c.1022G>A, p.(Arg341His)	19%	937X	3
KAT6A	17	NM_006766.4	c.5303C>T, p.(Ala1768Val)	17%	809X	3
NCOR2	25	NM_006312.5	c.3178del, p.(Arg1060Valfs*3)	14%	380X	3
PRKDC	69	NM_006904.6	c.9554G>A, p.(Arg3185Gln)	14%	409X	3
CDK12	2	NM_016507.3	c.1139G>A, p.(Arg380His)	14%	609X	3
KMT2D	11	NM_003482.3	c.3161C>T, p.(Pro1054Leu)	11%	849X	3
RSPO2	3	NM_178565.4	c.205C>T, p.(Arg69Cys)	11%	1297X	3
ZFHX3	10	NM_006885.3	c.9589del, p.(Gln3197Serfs*44)	5%	552X	3
GLI1	8	NM_005269.2	c.821del, p.(Gly274Alafs*6)	5%	2521X	3

§ Pathogenicity classes according to ACGS (Association for Clinical Genetic Science): class 5, pathogenic; class 4, likely pathogenic; class 3, of uncertain significance.
Estimated tumor cell content: 50%.
Mutation nomenclature: according to HGVS (Human Genome Variation Society).

(P 104) **COMPREHENSIVE IMMUNE PROFILING UNVEILS A SUBSET OF LEIOMYOSARCOMA WITH HOT TUMOR IMMUNE MICROENVIRONMENT**

Xiaolan Feng, MD PhD[1]; Laurie Tonon, NA, PhD[2]; Haocheng Li, PhD[3]; Elodie Darbo, PhD[4]; Erin Pleasance, NA, PhD[5]; Nicolas Macagno, MD PhD[6]; Armelle Dufresne, MD PhD[7]; Mehdi Brahmi, MD PhD[8]; julien BOLLARD, PhD[9]; francoise ducimetiere, NA, PhD[9]; Marie Karanian, MD PhD[8]; Alexandra Meurgey, MD PhD[9]; Gaëlle Perot, PhD[10]; Thibaud Valentin, MD[11]; Frederic Chibon, PhD[10]; Jean-Yves Blay, MD, PhD[12], (1) Tom Baker Cancer Center, Calgary, AB, Canada, (2) Centre Léon Bérard, lyon, Rhone-Alpes, France, (3) University of Calgary, Calgary, AB, Canada, (4) Université de Bordeaux, Bordeaux, Aquitaine, France, (5) BC Cancer, Vancouver, BC, Canada, (6) Aix Marseille Univ, Marseille, Rhone-Alpes, France, (7) CENTRE LEON BERARD, LYON, Rhone-Alpes, France, (8) CENTRE LEON BERARD AND CANCER RESEARCH CENTER OF LYON (INSERM U1052-CNRS5286), LYON, Rhone-Alpes, France, (9) Centre Léon Bérard, Lyon, Rhone-Alpes, France, (10) Cancer Research Center of Toulouse, Toulouse, Midi-Pyrenees, France, (11) Oncopole Claudius Regaud, Toulouse, Midi-Pyrenees, France, (12) Centre Léon-Bérard, Lyon, Rhone-Alpes, France

Objective: To investigate the immune biomarker in Leiomyosarcoma (LMS) which is a rare and recognized as an “immune-cold” cancer showing a poor response rate (< 10%) to immune checkpoint inhibitors (ICIs). However, durable response and clinical benefit to ICIs observed in few LMS, including, but not only, LMS with tertiary lymphoid structure (TLS) structures.

Methods: We used comprehensive transcriptomic profiling and a deconvolution method extracted from RNA-sequencing gene expression data in two independent LMS cohorts, the International Cancer Genome Consortium (ICGC, N=146) and The Cancer Genome Atlas (TCGA, N=75) to explore tumor immune microenvironment (TIME) in LMS.

Results: Unsupervised clustering analysis using the previously validated two methods-- 90 gene signature and the Cell-type Identification By Estimating Relative Subsets of RNA Transcripts (CIBERSORT), identified immune hot (I-H) and immune high (I-Hi) LMS respectively in ICGC cohort. Similarly, immune active groups (T-H, T-Hi) were identified in TCGA cohort using these two methods. These immune active (“hot”) clusters were significantly associated, but not completely overlapping, with several validated immune signatures such as sarcoma immune class (SIC) classification and TLS score, T cell inflamed signature (TIS) score, immune infiltration score (IIS), and macrophage score (M1/M2), with more patients identified by our clustering as potentially immune hot.

Conclusion: Comprehensive immune profiling revealed a subset of LMS with a distinct active ("hot”) TIME, consistently associated with several validated immune signatures in other cancers. This suggests that the methodologies that we used in this study warrant further validation and development, which can potentially help refine our current immune biomarkers to select the right LMS patients for ICIs in clinical trials.

N/A

Table 1. Patient and tumor characteristics were described in ICGC (n=111 patients) and TCGA cohorts (n=74 patients). Please note that the denominator for percentage calculation for last 3 roles-- first metastatic site, locoregional treatment for metastatic disease and systemic treatment for metastatic disease is the numbers of patients with metastatic disease (n=62), not the numbers of whole cohort (n=111) in ICGC cohort. * represents that 3 patients had neoadjuvant chemotherapy,16 patients had adjuvant chemotherapy, 3 patients had chemotherapy with palliative intent due to the extent of primary disease. ** represents that 2 patients had neoadjuvant radiotherapy. *** represents that 13 patients had debulking surgery, 3 patients had cryotherapy, 5 patients had radiofrequency treatment, 9 patients had radiotherapy either alone or combined with any of aforementioned treatments.

Patient/Tumor Characteristics	ICGC Cohort (n=111)	TCGA Cohort (n=74)
Age Median (min – max)	 64 (22-85)	 60 (33-90)
Gender Female Male	 84 (75.7%) 27 (24.3%)	 40 (54.1%) 34 (45.9%)
Tumour Size (cm) ≤ 5 > 5 and ≤ 10 >10 Unknown	 27 (24.3%) 43 (38.7%) 40 (36%) 1 (0.9%)	Not available
Tumor Grade (/FRSCC) 1 2 3 Unknown	14 (12.6%) 38 (34.2%) 52 (46.8%) 7 (6.3%)	 3 (4.1%) 54 (73%) 17 (23%) 0 (0%)
Tumour Location Limb Internal trunk Trunk wall Head and neck Gynecological Others	 20 (18%) 56 (50.5%) 10 (9%) 5 (4.5%) 16 (14.4%) 4 (3.6%)	24 (32.4%) 50 (67.6%)
Tumour Depth Superficial Deep	 8 (7.2%) 103 (92.8%)	Not available
Tumor Multifocality No Yes	108 (97.3%) 3 (2.7%)	Not available

(P 105) STING THERAPY INDUCES IMMUNOGENIC ANTI-TUMOR RESPONSES THAT ARE INDEPENDENT OF TUMOR STING EXPRESSION IN THE KP MODEL OF SOFT TISSUE SARCOMA

Karys M. Hildebrand, MD/PhD Candidate; Kurt N. Hildebrand, PhD Candidate; Carolina Salazar-Arcila, MSc; Michael Monument, MD MSc FRCS(C), University of Calgary, Calgary, AB, Canada

Objective: Undifferentiated pleomorphic sarcoma (UPS), is one of the most common, aggressive, and metastatic soft tissue sarcomas (STS) in adults. Systemic therapies against

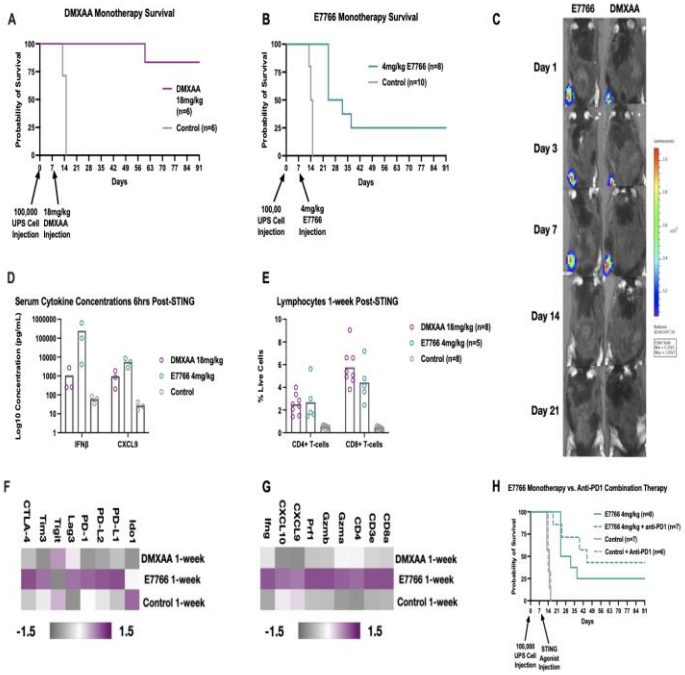
UPS are lacking and STS tumors are insensitive to most immunotherapies, including immune checkpoint blockade therapy. Generally, the tumor microenvironment (TME) of many STS, including UPS, are characteristically low in tumor infiltrating lymphocytes (TILs) and contain an abundance of tumor associated macrophages and myeloid derived suppressor cells. In previous studies, intratumoural (i.t.) activation of the STimulator of INterferon Genes (STING) pathway, using the murine specific agonist DMXAA, recruited TILs into the TME in the murine KP model of UPS and induced survival in 50-75% of treated mice. To assess the clinical translation feasibility of this immunotherapeutic strategy, investigating agonists capable of activating both human and murine STING must be evaluated. As a mechanism of evading the immune system's ability to identify and destroy cancer cells, many cancers have evolved to downregulate STING expression or have defective STING pathway signaling abilities. Although STING immunotherapy can induce immunogenic tumor clearance, it is unclear which cell types are critical contributors to this outcome, and if STING activation within tumor cells is conducive to this anti-tumor phenotype. To determine whether tumor STING signaling contributes to STING-mediated tumor clearance, STING therapy must be assessed in UPS tumor cell lines that do and do not engage in competent STING pathway signaling. Therefore, the objectives of this study are 1) to evaluate the anti-tumour potential of and TME modulation of the translational STING agonist, E7766 and 2) to characterize the importance of STING expression in host and UPS cells in the context of E7766 therapy.

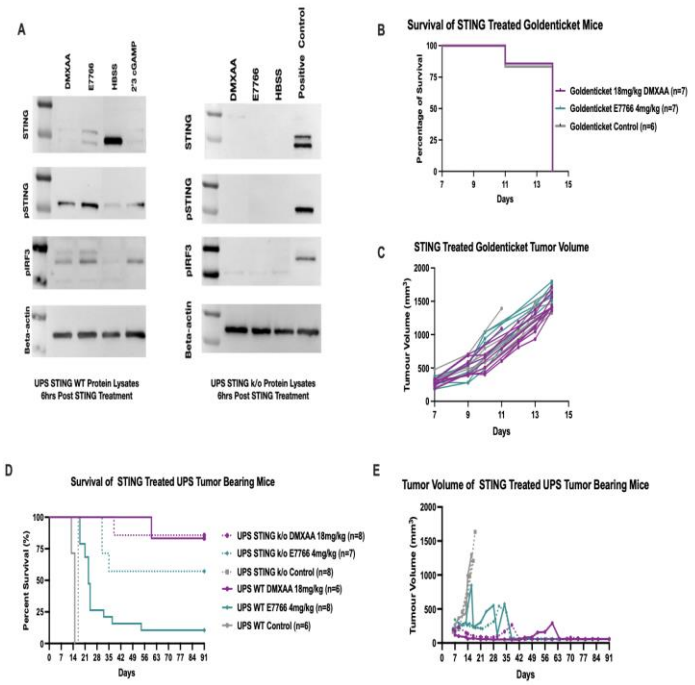
Methods: Immune competent C57Bl/6 mice were orthotopically engrafted with a syngeneic murine KP UPS cell line in the right hindlimb muscle. Once palpable, mice were treated with 4mg/kg of E7766 or 18mg/kg of DMXAA i.t. Following treatment, mice were divided into STING monotherapy or STING + anti-PD1 immune checkpoint blockade therapy groups which were followed for survival. Another cohort of DMXAA or E7766 treated mice were sacrificed at various time points to 1) evaluate the systemic effects of STING therapy using a Luminex 45-plex assay or 2) assess the impacts of STING therapy on the TME using flow cytometry and transcriptomics. Tumour volume measurements and bioluminescence were measured over time during survival studies. Any surviving mice were re-challenged with UPS in the contralateral limb. To assess STING expression in our UPS STING wildtype cells and to confirm that STING was knocked out of our UPS cell line, we completed western blots probing for STING and known proteins that are phosphorylated downstream of STING. To understand the role of STING expression in host cells, mice lacking the Sting1 gene (Goldenticket mice) were engrafted with UPS cells, treated with 4mg/kg of E7766 or 18mg/kg of DMXAA i.t, and followed for survival. Cells were treated with equimolar amounts of E7766 or DMXAA and were compared to an HBSS control. To determine the importance of UPS STING expression, immune competent C57Bl/6 mice were engrafted with UPS cells that have been genetically modified to lack Sting1 expression and treated with 4mg/kg of E7766 or 18mg/kg of DMXAA i.t and followed for survival.

Results: Of mice treated with E7766 monotherapy, 25% cleared their primary UPS tumors (Fig 1B). Mice who cleared their initial UPS tumors following E7766 treatment were re-challenged with an additional inoculation of UPS cells and displayed 100% clearance, indicating that adaptive immune protection was conferred (Fig 1 C). Interestingly, E7766 induced the greatest systemic production of interferon beta and CXCL9 at 6hrs following therapy relative to control and DMXAA treated groups (Fig 1D). Flow cytometry studies of tumors 1-week following STING therapy displayed that E7766 induces similar lymphocyte infiltration signatures to DMXAA (Fig 1E&F). Moreover, E7766 appeared to induce a greater transcriptomic enrichment of markers associated with negative regulation of immune responses, including PD-1/PD-L1 (Fig 1G). Excitingly, in the E7766 + anti-PD1 combination therapy group, survival increased as 42% of treated mice eradicated their UPS tumors (Fig 1H). Western blots of protein lysates from UPS STING WT and UPS STING k/o cell lines showed that in response to E7766 and DMXAA

treatment, the WT cell line expresses STING and both STING and IRF3 are phosphorylated in response to STING therapy (Fig 2A). Conversely, these observations are abrogated in the UPS STING k/o blots (Fig 2A). In vivo experiments evaluating the contributions of STING expression in host and tumor cells, we found that host STING expression was required to observe the survivor phenotype in both DMXAA and E7766 treated mice (Fig 2B&C). Moreover, mice bearing STING k/o UPS tumors demonstrated an improved survival phenotype in response to E7766 therapy, as 57% of treated mice eradicated their tumors (Fig 2D&E). Single cell mRNA sequencing experiments will be completed to determine which cell types in the UPS TME are key contributors to STING signalling following therapy.

Conclusion: We have shown that E7766 mediated STING activation is a promising immunotherapeutic strategy for UPS in our murine model. E7766 therapy can induce tumor clearance and adaptive immune protection against UPS re-challenge. Excitingly, E7766 STING therapy can also sensitize our model to anti-PD1 ICB therapy. We have shown that therapeutic responses to STING agonist therapies are dependent on host STING expression and not tumor STING expression. In fact, UPS tumors deficient in STING may show improved therapeutic responses as our data currently suggests that UPS tumors lacking STING expression respond better to E7766 therapy. Ultimately, this study demonstrates the potential opportunity for clinical translation of STING as an immunotherapy for STS which could significantly improve outcomes for this patient demographic.





(P 106) **CHARACTERIZATION OF AN IMMUNE COMPETENT, MURINE MODEL OF SOFT TISSUE SARCOMA**

Kurt N. Hildebrand, PhD Candidate[1]; Karys M. Hildebrand, MD/PhD Candidate[1]; Carolina Salazar-Arcila, MSc[1]; Arvind Singla, PhD[2]; Franz J. Zemp, PhD[1]; Michael Monument, MD MSc FRCS(C)[1], (1) University of Calgary, Calgary, AB, Canada, (2) McCaig Institute for Bone and Joint Health, Calgary, AB, Canada

Objective: Soft tissue sarcomas (STS) are rare and aggressive tumors that are notoriously difficult to treat as they rarely respond to conventional systemic therapies and require extensive operations. To achieve a stronger understanding of STS biology and to investigate new therapeutic interventions for this disease, more pre-clinical, immune competent models must be developed. Our laboratory has developed a new murine model of STS through lenti-viral cre-recombinase mediated gene knockouts of PTEN and p53, and activation of the KRAS oncogene (Trp53fl/fl; Ptenfl/fl; KrasG12D/+). Herein, we describe the histological characteristics and immune infiltrates of this novel STS in comparison to the previously published KP model of UPS.

Methods: The Trp53fl/fl; Ptenfl/fl; KrasG12D/+ mice were generated by breeding B6.129P2-Trp53tm1Brn/J (Trp53fl), B6.129S4-Krastm4Tyj/J (KrasG12D), and B6.129S4-Ptentm1Hwu/J mice. Once mice reached 6-8 weeks of age, 10µl of 1x10^8 pfu a CMV-driven cre-recombinase plasmid (lenti-cre) was administered in the deep margin on the tibialis anterior muscle of the right hindlimb following proximal-lateral tibia periosteal scratch with a 25-gauge needle to induce spontaneous tumor development. A female spontaneous primary STS tumor was processed to develop the cell line, KPP1053. Syngeneic tumors were developed through the orthotopic engraftment of 100,000 KPP1053 cells in the hindlimb (Fig. 1). As a control murine

STS model for comparison, we will use Trp53fl/fl; KrasG12D/+ (KP) that our laboratory has previously characterized an immune competent murine model of STS through mutations to Trp53fl/fl; KrasG12D/+. The KP model is characterized as having an abundance of myeloid cells, with few tumor infiltrating lymphocytes, and poor response to immunotherapy. Spontaneous and cell line KP tumors are developed using the same strategy as above and used controls in comparison to spontaneous Trp53fl/fl; Ptenfl/fl; KrasG12D/+ and syngeneic KPP1053 tumors, respectively. Trp53fl/fl; Ptenfl/fl; KrasG12D/+, KPP1053, and KP spontaneous and syngeneic tumors were harvested for flow cytometry, histology, and mRNA sequencing at a tumor volume of 500-800mm^3. Assays to assess the systemic cytokine signatures of tumor bearing mice are currently taking place.

Results: Spontaneous tumors developed in 23/25 mice within 22-62 days of lenti-cre injection, which is significantly faster than lenti-cre induced KP tumors ($p < 0.0001$; Fig. 2A). Of mice that were orthotopically engrafted with KPP1053 cells, 100% developed primary STS tumors within 7 days of engraftment reaching humane endpoint by day 14, which is similar to KP cell line derived tumors (Fig. 2B). All spontaneous tumors retained similar histological features, with negative cytokeratin staining, focal or patchy desmin staining, and positive smooth muscle actin staining (Figs. 2C-E). In assessing immune infiltrates, flow cytometry revealed that macrophages and dendritic cells were similar across tumor models (Figs. 3A & B). However, KPP1053 syngeneic tumors appeared to have an elevated infiltration of neutrophils (Fig. 3C). Additionally, Trp53fl/fl; Ptenfl/fl; KrasG12D/+ spontaneous tumors had higher proportions of CD8+ T-cells compared to the KPP1053 syngeneic tumors (Fig. 3D), but no differences in CD4 T cells (Fig. 3E). However, in comparing KP syngeneic tumours to KPP1053 syngeneic tumors, there were no differences in lymphocytes, which was also shown for KP and Trp53fl/fl; Ptenfl/fl; KrasG12D/+ spontaneous tumors.

Conclusion: We have generated a new immune competent murine model of STS. This new model shows similar levels of myeloid cells and lymphocytes compared to the KP model of STS. Thus, this provides another murine STS model to assess pre-clinical treatments. This allows for more models to improve generalizability of study findings. Future experiments assessing the efficacy of immune checkpoint inhibitor immunotherapy and response to radiation therapy are ongoing.

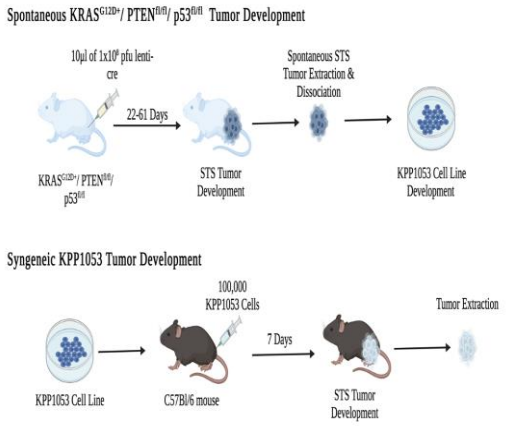


Figure 1. Tumor development workflow. Strategy for developing *Trp53$^{fl/fl}$; Pten$^{fl/fl}$; Kras$^{G12D/+}$* (**A**) spontaneous and (**B**) cell line (syngeneic) tumors.

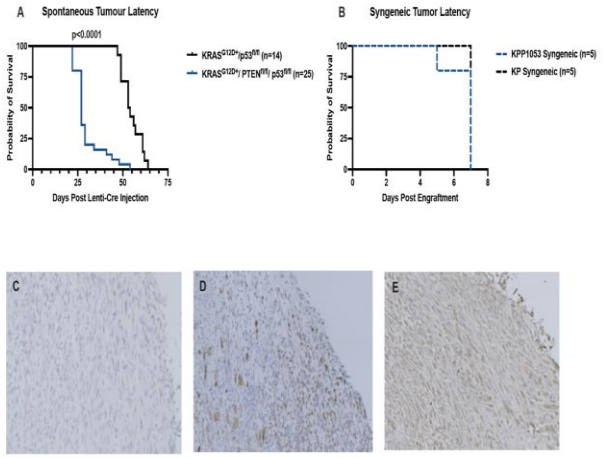


Figure 2. Growth kinetics of STS tumors & immunohistological characteristics. (A) Spontaneous *Trp53*$^{fl/fl}$*; Pten*$^{fl/fl}$*; Kras*$^{G12D/+}$ tumors develop between days 22-62, whereas spontaneous *Trp53*$^{fl/fl}$*; Kras*$^{G12D/+}$ tumors develop between days 54-61. **(B)** Syngeneic *KPP1053* tumors develop on a similar timeline to KP syngeneic tumors. Immunohistochemistry staining of tumors for **(C)** cytokeratin, **(D)** desmin, and **(E)** smooth muscle actin.

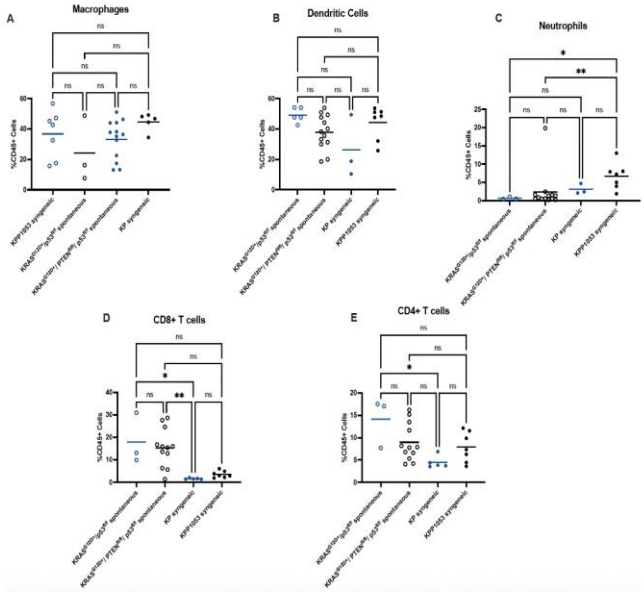


Figure 3. Flow cytometry quantification of myeloid and lymphocytes within STS tumors. Myeloid cell analysis assessing **(A)** macrophages, **(B)** Dendritic cells, and **(C)** Neutrophils. Lymphocyte analysis assessing **(D)** CD8 and **(E)** CD4 T cells. Kruskal-Wallis tests used to compare groups. *p<0.05, **p<0.01.

(P 107) TRABECTEDIN AUGMENTS ONCOLYTIC HERPES SIMPLEX VIROIMMUNOTHERAPY EFFICACY IN PRECLINCAL BONE SARCOMA MODELS THROUGH ENHANCED IMMUNE-MEDIATED MECHANISMS

Emily M. Franz Ringwalt, BS[1]; Mark A. Currier, MS[2]; Andrea M. Glaspell, BS[2]; Chun-Yu Chen, PhD[2]; Matthew Cannon, PhD[2]; Amy C. Gross, MS[2]; Maren Cam, PhD[2]; Pin-Yi Wang, PhD[2]; Ryan D. Roberts, MD, PhD[2]; Timothy P. Cripe, MD, PhD[2], (1) The Ohio State University/Nationwide Children's Hospital, Columbus, OH, (2) Nationwide Children's Hospital, Columbus, OH

Objective: Treatment of patients with advanced cases of osteosarcoma, the most common pediatric bone sarcoma, remains largely unsuccessful. Decades of research yielded little improvement to the treatment regimens for patients affected by this bone sarcoma. We aim to improve patient outcomes through the use of oncolytic herpes simplex viroimmunotherapy, which induces tumor cell-specific lysis and anti-tumor immune activation. After observing the impressive synergy between oncolytic herpes simplex viroimmunotherapy and the FDA-approved drug trabectedin, we sought to determine the mechanisms of this efficacy. Previous results indicated that treatment of immunodeficient human Ewing sarcoma xenograft models may depend on enhanced oncolytic herpes simplex viroimmunotherapy spread through trabectedin-mediated disruption of tumor intrinsic antiviral gene expression. However, due to species-dependent differences, mouse syngeneic models do not display sufficient viral spread for this mechanism of synergy. Thus, based on the known ability of trabectedin to reduce immunosuppressive monocytes and macrophages, we hypothesized that the observed synergy is immune-mediated in immunocompetent osteosarcoma tumor models.

Methods: In multiple preclinical syngeneic mouse models of osteosarcoma, we combined oncolytic herpes simplex viroimmunotherapy with trabectedin to determine the efficacy and survival benefits of the combination therapy with and without immune effector populations. We employed single-cell RNA sequencing and flow cytometry to probe the synergistic mechanism in immunocompetent osteosarcoma models based on differences in immune infiltrate and polarization between treatment groups.

Results: Multiple models of osteosarcoma treated with trabectedin combined with oncolytic herpes simplex viroimmunotherapy showed impressive synergy, with the majority displaying complete or partial tumor regressions. The combination therapy exhibited significant survival benefits ($p < 0.05$) alongside low average tumor burdens across immunocompetent models. Single-cell RNA sequencing data showed decreased immunosuppressive, M2-like gene expression in monocytes and macrophages in combination-treated tumors compared to those treated with the viroimmunotherapy alone. Flow cytometry also supported a decrease in M2-like macrophages in combination-treated tumors. In correlation with these observations, we detected enhanced granzyme gene expression in NK and CD8 T cells from combination-treated tumors as compared to monotherapy-treated tumors, thereby displaying potential anti-tumor immune activation. Indeed, the depletion of NK cells or CD8 T cells caused a loss of the combination efficacy, indicating that NK or CD8 T cells are necessary for combination efficacy. Interestingly, the depletion of CD4 T cells resulted in increased efficacy compared to the combination control, correlating with single-cell RNA sequencing data that nearly all Cd4+ T cells in the tumor microenvironment are Foxp3+ T regulatory cells.

Conclusion: Our results indicate that trabectedin enhances oncolytic herpes simplex viroimmunotherapy in preclinical immunocompetent osteosarcoma models through immune-mediated anti-tumor mechanisms. Trabectedin removes the immunosuppressive barriers of

anti-inflammatory, pro-tumor monocytes and macrophages and frees immune effector cells, namely NK and CD8 T cells, to induce tumor cell death. This immune-mediated mechanism may synergize with our observation of increased viral spread in human tumors to generate greater combinatorial efficacy in patients.

(P 108) EFFECT OF TUMOUR-TARGETED HYPERTHERMIA AND CHEMOTHERAPY ON THE IMMUNE PROFILE OF A MURINE MODEL OF EMBRYONAL RHABDOMYOSARCOMA

Julia Nomikos, BSc[1]; Claire Wunker, MD, MSc[2]; Adam C. Waspe, PhD[3]; Yael Babichev, PhD[4]; Karolina Piorkowska, MSc[3]; Suzanne M. Wong, MASc[3]; Warren Foltz, MSc, PhD[5]; J Ted Gerstle, MD, FRCSC, FACS[6]; Elizabeth Demicco, MD, PhD[7]; Abha Gupta, MD MSc[8]; Cynthia J. Guidos, PhD[9]; James M. Drake, BSE, MBBCh, MSc, FRCSC, FACS[3]; Rebecca Gladdy, MD, PhD[10], (1) Lunenfeld-Tanenbaum Research Institute - Mount Sinai Hospital, Toronto, ON, Canada, (2) Saint Louis University, St. Louis, MO, (3) Hospital for Sick Children, Toronto, ON, Canada, (4) Sinai Healthcare System, Toronto, ON, Canada, (5) Princess Margaret Hospital, University Health Network, Toronto, ON, Canada, (6) Memorial Sloan Kettering Cancer Center, New York, NY, (7) Sinai Health System, Toronto, ON, Canada, (8) Princess Margaret Cancer Center, Toronto, ON, Canada, (9) Hospital for Sick Children Research Institute, Toronto, ON, Canada, (10) Mount Sinai Hospital, Sinai Health System, and Princess Margaret Cancer Centre Toronto, ON, Canada Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada University of Toronto, Toronto, ON, Toronto, ON, Canada

Objective: Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma. Prognosis remains poor in patients with recurrent/relapsed RMS, and treatment-associated toxicity is prevalent. Current standard of care chemotherapy includes vincristine, while second line chemotherapy includes doxorubicin which is associated with dose-limiting cardiotoxicity. Thermosensitive liposomal doxorubicin (TLD) releases doxorubicin at a temperature of approximately 40-42°C. To heat the drug in vivo, magnetic resonance-guided high intensity focused ultrasound (MRgHIFU) can produce tumour-localized hyperthermia (HT) to target doxorubicin release to the site of disease. HT+TLD treatment resulted in improved survival and reduced off-target toxicity in an immunocompetent embryonal RMS (ERMS) mouse model. HT has also been shown to modulate the immune response, which may allow for a synergistic effect with immunotherapies. The objectives of this project are to characterize the immune profile of a murine model of ERMS over time and to determine what effect chemotherapy and/or HT has on immune infiltrates.

Methods: C57BL/6 mice were intramuscularly injected with a murine ERMS cell line into the right hind limb to generate tumours. Mice were divided into 2 groups: HT or normothermia (NT). Tumours of HT mice were heated to 40.5°C for 21.5 minutes, while NT mice received no heat treatment. Drugs tested included vincristine (VINC), free doxorubicin (FD), and TLD resulting in 8 treatment groups: control (untreated), VINC, FD, TLD, HT, HT+VINC, HT+FD, and HT+TLD. Tumours were collected at 24 and 168 hours post-treatment (hpt). Immunohistochemistry for CD11b (myeloid immune cells), CD3 (T cells), and B220 (B cells) was conducted in n=99 tumours and quantified using HALO image analysis software. Imaging mass cytometry (IMC) for a panel of 17 markers was conducted for central and peripheral 1mm2 regions of interest in a subset of 3 tumours: untreated, HT 24 hpt, and HT 168 hpt, along with a mouse spleen used as a control.

Results: Of the three immunohistochemical markers, CD11b+ cells were the most abundant, followed by T cells, and very few B cells, which have been associated with increased survival and immunotherapy response in sarcoma. TLD alone decreased CD11b+ and T cells within tumours at 24 hpt (p=0.0032, 0.0137 respectively) (Figure 1). Vincristine also decreased CD11b+ cells within tumours at 168 hpt (p=0.0338) (Figure 1). HT increased CD11b+ cells in TLD treated tumours at 24 hpt, and increased B cells in tumours treated with no drug at 168 hpt (p=0.0101, 0.0489 respectively) (Figure 2). IMC revealed that the most abundant immune-related markers within tumours were MHC II, Sirpa, CD11b, and CD64, while the least abundant were CD8a, B220, CD22, and Ly6G (Figure 3). Comparison of IMC between differently treated tumour samples suggested central clustering of MHC II, increased CD31, increased Ly6G, increased peripheral CD64, and decreased dendritic cells (Cd11c+, CD64-) in the HT 24 hpt tumour compared to the untreated tumour (Figure 4).

Conclusion: The tumour microenvironment in this murine model of ERMS is macrophage dominated, with very few cytotoxic T cells, B cells, and neutrophils. Drug treatment alone can impact the immune microenvironment, with both TLD and VINC causing decreases in myeloid immune cell and T cell quantity in tumours at either 24 or 168 hpt. The mechanism behind this is under investigation. HT may be stimulating an immune response, particularly with respect to increased macrophages, neutrophils, and endothelial cells at 24 hpt, increased B cells at 168 hpt, and decreased dendritic cells (potentially due to migration to the lymph nodes for antigen presentation) at 24 hpt. Ultimately, this data will help us understand how we can harness HT-related immune stimulation from an already promising therapy (HT+TLD) to create an even greater synergistic effect with immunotherapy in order to increase treatment efficacy while reducing toxicity in RMS patients.

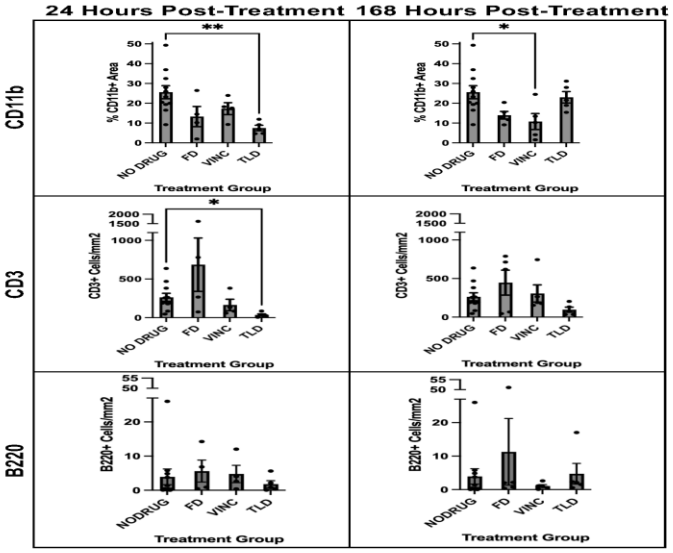


Figure 1. Comparison of CD11b, CD3, and B220 staining of whole cross sections of normothermia drug treated tumors 24 hours and 168 hours post-treatment (FD – free doxorubicin, VINC – vincristine, TLD –thermosensitive liposomal doxorubicin). Scale bars vary between graphs. Total n=50. Error bars: mean ± SEM. Statistics: Kruskal-Wallis test with Dunn's multiple comparisons test. * indicates significance with a p value < 0.05, ** indicates significance with a p value < 0.01. CD11b 24h NO DRUG/TLD: p=0.0032. CD11b 168h NO DRUG/VINC: p=0.0338. CD3 24h NO DRUG/TLD: p=0.0137.

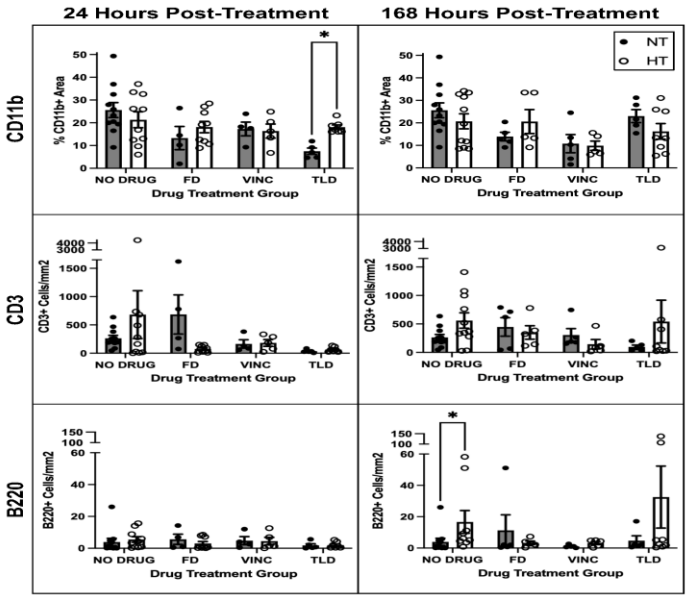


Figure 2. Comparison of the effect of hyperthermia on CD11b, CD3, and B220 staining of whole cross sections of treated tumors 24 hours and 168 hours post-treatment (FD - free doxorubicin, VINC - vincristine, TLD -thermosensitive liposomal doxorubicin). Hyperthermia (HT) treated tumours (white bars), normothermia (NT) tumours (grey bars). Scale bars vary between graphs. Total n=99. Error bars: mean ± SEM. Statistics: multiple Mann-Whitney tests with Holm Sidak multiple comparison's test. * indicates significance with a p value < 0.05. CD11b 24h TLD/HT+TLD: p=0.0101. B220 168h NO DRUG/HT: p=0.0489.

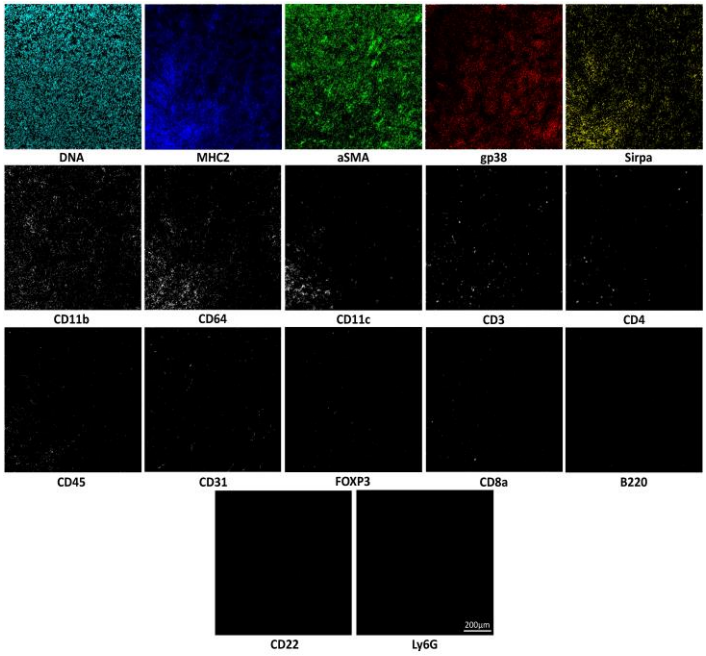


Figure 3. Imaging mass cytometry of a 1mm² region of interest in the periphery of an untreated tumour. Scale bar applies to all images and highest density stains are pseudo coloured.

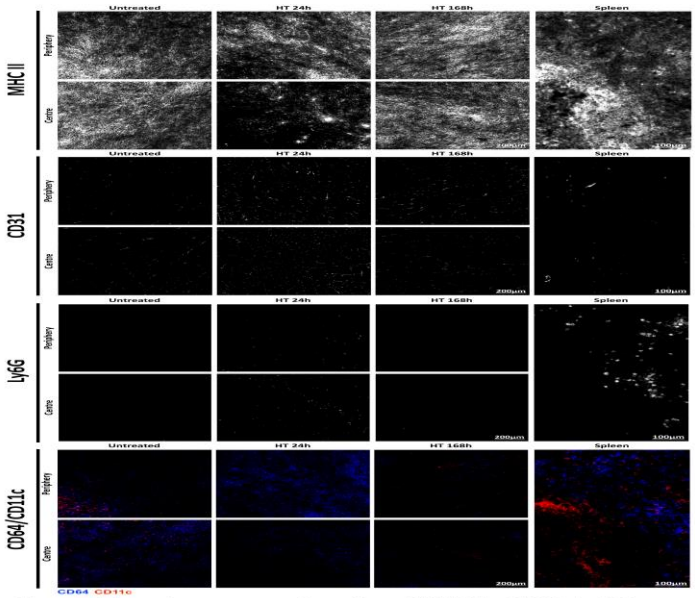


Figure 4. Imaging mass cytometry of MHC II, CD31, Ly6G, CD64 and CD11c markers of central and peripheral tumour regions of interest (ROI) in an untreated, hyperthermia (HT) 24 hour post-treatment, and HT 168 hour post-treatment tumour. Positive staining is indicated in white, with the exception of CD64 (blue) and CD11c (red). Periphery: an ROI within 1mm of the tumour's edge. Centre: an ROI further than 1mm from the tumour's edge. Scale bar for tumour cross sections are all the same.

(P 109) INTENSE PRECLINICAL ACTIVITY BY B7H3-SPECIFIC CAR.CIK LYMPHOCYTES AGAINST EPITHELIOID SARCOMA

Alessandra Merlini, MD, PhD[1]; Annamaria Massa, PhD[2]; Valentina Zuco, N/A, MSc[3]; Noemi Arrighetti, PhD[3]; Chiara Donini, PhD[2]; Letizia Vitali, BSc[2]; Silvia Brusco[2]; Martina Rabino, MD[2]; Alessia Proment, BSc[2]; Federica Galvagno, BSc[2]; Andrea Lombardi, BSc[2]; Elisa Vigna, PhD[2]; Elisa Landoni, PhD[4]; Gianpietro Dotti, PhD[4]; Giovanni Grignani, MD[5]; Silvia Novello, MD, PhD[6]; Lorenzo D'Ambrosio, MD[7]; Nadia Zaffaroni, PhD[8]; Valeria Leuci, PhD[2]; Sandro Pasquali, MD, PhD[9]; Dario Sangiolo, MD, PhD[2], (1) Department of Oncology, University of Turin, Torino, Piemonte, Italy, (2) Department of Oncology, University of Turin, Turin, Piemonte, Italy, (3) Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Lombardia, Italy, (4) UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC, (5) Candiolo Cancer Institute, FPO – IRCCS, Turin, Piemonte, Italy, (6) Department of Oncology, San Luigi Hospital, University of Turin, Orbassano, Piemonte, Italy, (7) University if Turin, Turin, Italy, Torino, Piemonte, Italy, (8) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (9) Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy

Objective: Epithelioid sarcoma is an ultra-rare mesenchymal tumor characterized by either genetic or epigenetic loss of INI1/SMARCB1 function. This event leads to constitutive EZH2 activation, promoting tumorigenesis epigenetically. In advanced stages, epithelioid sarcoma is characterized by dismal prognosis and limited therapeutic options. The advent of EZH2 inhibitors has revived hope for advanced epithelioid sarcoma; however, as seen with the majority of targeted treatments, resistance typically ensues. Hence, epithelioid sarcoma represents a relevant unmet medical need; reliable preclinical studies for the development of innovative, effective treatments are awaited. Purpose of this study is to explore and define a

preclinical model for a novel CAR-based cellular immunotherapy against epithelioid sarcoma, including the possible targeting of sarcoma cell subsets with stemness features that are emerging as relevant components for chemoresistance and disease relapse in several solid tumors. The strategy is focused on Cytokine-induced Killer lymphocytes (CIK), ex vivo expanded T-NK lymphocytes endowed with intrinsic HLA-independent anti-tumor activity (NKG2D mediated). CIK will be redirected with a chimeric antigen receptor (CAR) against B7 homolog 3 protein (B7-H3), an emerging target expressed by multiple solid tumors and associated with immunosuppressive functions and stemness.

Methods: The experimental setting is based on patient-derived CAR.CIK and 2 proximal epithelioid sarcoma (EpS) cell lines (ES1, ES2) with confirmed loss of INI1/SMARCB1, derived from two patients affected by advanced EpS. CAR.CIK were generated by engineering circulating PBMC precursors with a retroviral vector encoding for a 2nd generation anti-B7-H3 CAR with CD28 co-stimulatory domain and ex vivo expanded (3 weeks) according to a standard protocol. Tumor killing activity by CAR.CIK was assessed by coculture in vitro (48h) and analyzed by flow cytometry. To visualize and track the fate of putative sarcoma cancer stem cells (sCSCs), EpS cells were engineered with a lentiviral CSC-detector wherein the promoter of stem-gene Oct4 controls the expression of eGFP, previously validated in sarcomas (Sangiolo et al, Cancer Research 2014; Oncoimmunology 2018).

Results: We effectively ex vivo generated CAR.CIK from patients (n=3). The membrane expression of anti-B7H3 CAR ranged from 50 to 80%. The main phenotype of mature CAR.CIK was:CD3+CD56+=30% CD3+CD8+: 70%, NKG2D= 60%, PD-1=15%), comparable to paired unmodified control (NTD.CIK). The CAR-target B7-H3 was confirmed to be intensely expressed by our ES cell lines (85-90%), along with NKG2D ligands (MIC A/B >80%), while a modest expression of both PD-L1 (10-20%) and PD-L2 (10-15%) was detected. The tumor killing activity in vitro of anti-B7-H3 CAR.CIK against ES was intense and significantly superior as compared with control NTD-CIK, especially at very low effector/target (E/T) ratios, ideally more representative of hypothetical clinical scenarios (95% vs 68% E/T=10:1; 70% vs 20% E/T= 1:1, 60% vs 2% E/T=1:4, $p< 0.005$ n=4). Within ES cells, we could visualize a subset of putative GFP+ sarcoma CSCs (8-10% n=2) that were equally killed by B7H3 CAR.CIK.

Conclusion: We report first proof of concept of a CAR-based cellular immunotherapy against epithelioid sarcomas, including a subset of putative CSCs, underscoring B7-H3 as a relevant target in this challenging, ultra-rare soft tissue sarcoma histotype.

(P 110) GCAR1: GPNMB CAR T THERAPY FOR MIT FAMILY TRANSLOCATION POSITIVE CANCERS

Franz J. Zemp, PhD[1]; Louisa Guignard, MSc[1]; Yeon Suh, MSc[1]; Hongrui Liu, PhD[1]; Cini John, PhD[1]; Nicole Prokopishyn, PhD[2]; Sacha Benaoudia, PhD[1]; Potts Kyle, PhD[1]; Heewon Seo, PhD[1]; Kata Osz, n/a[3]; Jonathan Alex, n/a[4]; Laura Mah, n/a[1]; Zackariah Breckenridge, MSc[1]; Donna Senger, PhD[5]; Lisa Difrancesco, MD[1]; Travis Ogilvie, MD[1]; Jennifer Quizi, PhD[6]; Robert Holt, PhD[7]; John Bell, PhD[6]; Agnieszka Wozniak, PhD[8]; Michael Monument, MD MSc FRCS(C)[1]; Patrick Schöffski, MD, MPH[9]; Kiril Trpkov, MD, FRCPC[10]; Jennifer Chan, MD[1]; Sorana Morrissy, PhD[1]; Jan-Willem Henning, MBChB, FRCPC[3]; Shafey Mona, MD, FRCPC[3]; Douglas J. Mahoney, PhD[4], (1) University of Calgary, Calgary, AB, Canada, (2) Alberta Precision Laboratories, Calgary, AB, Canada, (3) Tom Baker Cancer Center, Calgary, AB,

Canada, (4) Univeristy of Calgary, Calgary, AB, Canada, (5) McGill Univeristy, Montreal, PQ, Canada, (6) University of Ottawa, Ottawa, ON, Canada, (7) University of British Columbia, Vancouver, BC, Canada, (8) Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Vlaams-Brabant, Belgium, (9) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium, (10) Alberta Precision Laboratories, Staff Pathologist, Calgary, AB, Canada

Objective: Motivated by a local AYA patient with recurrent, metastatic alveolar soft part sarcoma (ASPS), we have designed a novel Chimeric Antigen Receptor (CAR) T therapy targeting Glycoprotein NMB (GPNMB). GPNMB expression is regulated by members of the microphthalmia-associated transcription factor (MiT) family which includes TFE3, TFEB and MITF. In ASPS, translocation-positive renal cell carcinoma (tRCC), and some cases of epithelioid hemangiosarcoma (EHE), GPNMB is driven to high and homogenous levels by pathognomonic fusion proteins containing MiTF family members. Encouraged by GPNMB's low expression in normal tissue and ubiquitous expression in these cancers, we are proceeding into a first-in-person single patient study (SPS) that will expand to a pan-Canadian, multicenter Phase I.

Methods: GCAR1 is a novel, 2nd generation CAR T therapy containing a scFV targeting GPNMB and 41bb and CD3ζ T cell signalling domains. Preclinical testing utilized an ASPS patient-derived xenograft (PDX) and preclinically manufactured healthy donor or patient-matched CAR T cells. Clinical development of GCAR1 included producing a lentiviral product under principles of GMP in collaboration with the BC Cancer Agency and Biotherapeutics Manufacturing Centre at the Ottawa Hospital Research Institute. In-house manufacturing validation runs were performed by Alberta Precision Laboratory Cell Therapy Labs on the CliniMACS Prodigy® using healthy or patient-derived apheresis material. Phase I clinical trial design is currently being developed in collaboration with the Canadian Clinical Trials Group (CCTG).

Results: In preclinical testing, we found GCAR1 efficiently kills GPNMB-expressing ASPS and tRCC cells lines in culture, while resulting in complete and durable remissions when systemically delivered to orthotopic PDX mouse models of ASPS primary tumours and brain metastasis. In primary tumour models, GCAR1 expanded within the tumour microenvironment resulting in a >500-fold expansion in the blood without detectable toxicities. GCAR1 clinical development included three validation runs on the CliniMACS Prodigy®, all of which expanded functional GCAR1 CAR T cells that met standard release criteria. A first-in-person SPS of GCAR1 in a AYA ASPS patient with multiple, progressing pulmonary metastasis is nearing initiation. The SPS protocol will administer 1e6 GCAR1/kg with primary endpoints measuring CAR-related toxicity and efficacy. If available, preliminary results of the SPS to be presented at the meeting.

Conclusion: Preclinical evaluation of GCAR1 demonstrated robust anti-tumour activity against GPNMB-expressing cells in vitro and in vivo, which has led to the rapid translation of this therapeutic for an ASPS patient without further treatment options. These studies are informing the design of a CCTG-sponsored Phase I clinical trial indicated for all MiT-translation cancers.

(P 111) INTERIM ANALYSIS OF A PHASE 1 STUDY USING IL12-FHAB WITH OPTIMIZED PHARMACOKINETICS

Sant P. Chawla, MD, FRACP[1]; Victoria Chua, MD, CLS[1]; Erlinda M. Gordon, MD[2]; John Cini, PhD[3]; Susan Dexter, BS[3]; Manuel DaFonseca, BS[3]; Justus Bingham, BS[4]; Grantham W. Hogeland, PharmD[5]; Richard T. Kenney, MD[3], (1) Sarcoma Oncology Center, Santa Monica, CA, (2) Sarcoma Oncology Research Center, Santa Monica, CA, (3) Sonnet BioTherapeutics, Princeton, NJ, (4) Momentum Metrix, Omaha, NE, (5) Momentum Metrix, LLC, Omaha, NE

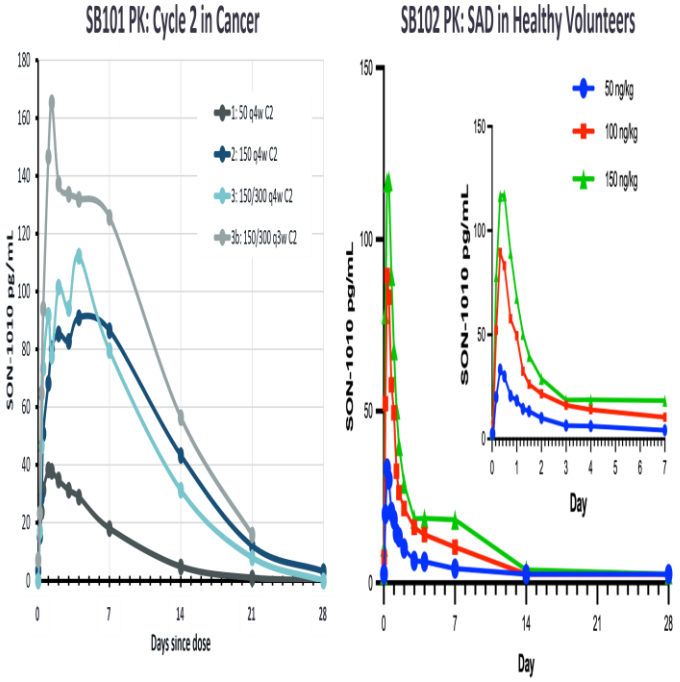
Objective: Recombinant interleukins (IL) have had limited clinical success due to inefficient tumor targeting and short pharmacokinetics (PK), requiring frequent dosing that leads to aberrant immunostimulation and toxicity. IL-12 is a promising cancer treatment due to its activation of T and natural killer (NK) lymphocytes to produce interferon-gamma (IFN-γ), yet dosing strategies have failed to provide adequate therapeutic benefit in humans. To address these issues, we developed a novel platform that delivers either mono- or bifunctional immunomodulator(s) linked to a Fully-Human, Albumin Binding scFv domain (FHAB®). The platform provides enhanced tumor targeting and retention through albumin binding to over-expressed FcRn, GP60, and SPARC, which results in an improved PK profile, in addition to a dose-sparing effect that decreases the toxicity risk, and provides a broader therapeutic index. Excellent tumor growth inhibition was seen using a “cold” immunosuppressive B16F10 melanoma model for comparing the efficacy of IL12-FHAB with rIL-12, resulting in significant increases in activated NK, NKT, Th1, and cytotoxic CD8 T cells.

Methods: We are conducting a first-in-human, dose-escalation trial, SB101, to evaluate the safety and tolerability of SON 1010 (IL12-FHAB) and to determine the maximum tolerated dose (MTD) in patients with advanced solid tumors (NCT05352750). The study has a traditional 3+3 design, modified to take advantage of the known tachyphylaxis of IL-12 with the introduction of a desensitizing first dose to allow administration of higher maintenance doses.

Results: No dose-limiting toxicities have been encountered in the first 3 dose cohorts and the MTD is at least 300 ng/kg. While some adverse events are similar to those reported in other studies of IL 12 at these doses, they have been transient and tolerable, allowing further dose escalation. Increases in IFN-γ were dose-related, controlled, and prolonged. The levels peaked at 24 to 48 hours and returned to baseline after 2 to 4 weeks. Low levels of IL-10 were induced in a dose-dependent manner. No drug-related increase was seen with IL-1β, IL-6, IL-8, or TNF-α and there has been no evidence of cytokine release syndrome. The preliminary geomean elimination half-life (T½) was 113 hours with single-compartment kinetics, compared with 12 hrs for SC rhIL-12. Interestingly, two-compartment kinetics were seen in a parallel single-ascending dose (SAD) study of SON-1010 in healthy volunteers. The accumulation estimates are within the margin of error and are not likely to be physiologically significant with subcutaneous dosing of SON-1010 every 3 weeks. Nine of 15 patients had stable disease at the first follow-up CT, 4 of whom were progressing at study entry. Five patients were stable at 4 months (36% clinical benefit) and 2 had unconfirmed progressive disease; 1 patient remains stable after 12 months on SON-1010 with evidence of tumor regression.

Conclusion: SON-1010 has been tolerated well with a delayed PK profile and causes a controlled and prolonged elevation of IFN-γ. The PK comparison with dosing in healthy volunteers suggests that SON-1010 is being retained in tumor tissue, as the FHAB platform was designed to do. This monofunctional immunotherapeutic drug candidate may have a positive synergistic effect with an immune checkpoint inhibitor (ICI), particularly with ‘cold’ tumors that over-express SPARC like sarcoma and ovarian cancer. The next stage of

development will be to explore the MTD of SON-1010 in combination with an ICI, then to compare that approach with the standard of care in Phase 2.



(P 112) PHASE 1/2 STUDY OF YH001, A CTLA-4 ANTIBODY WITH THE SUBCUTANEOUSLY ADMINISTERED PD-L1 ANTIBODY ENVAFOLIMAB, WITH OR WITHOUT DOXORUBICIN IN PATIENTS WITH ADVANCED OR METASTATIC SOFT TISSUE SARCOMA

Sant P. Chawla, MD, FRACP[1]; Seth Pollack, MD[2]; Richard F. Riedel, MD[3]; George Thomas Budd, MD[4]; Melissa A. Burgess, MD[5]; Alex Grint, Bachelors[6]; Bonne Adams, MBA[7]; Dongliang Zhuang, PhD[7]; James Freddo, MD[8]; Charles Theuer, MD, PhD[9]; Sujana Movva, MD[10], (1) Sarcoma Oncology Center, Santa Monica, CA, (2) Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL, (3) Duke Cancer Institute, Durham, NC, (4) Cleveland Clinic, Cleveland, OH, (5) University of Pittsburgh Medical Center Hillman Cancer Center, Pittsburgh, PA, (6) TRACON Pharmaceuticals INC., San Diego, CA, (7) TRACON Pharmaceuticals Inc, San Diego, CA, (8) TRACON Pharmaceuticals Inc, Jackson, WY, (9) TRACON, San Diego, CA, (10) Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Doxorubicin is the standard frontline chemotherapeutic used in sarcoma patients and has been well tolerated when combined with PD-(L)1 antibodies. Given the non-overlapping toxicity profiles of checkpoint inhibitors and chemotherapy, the addition of dual checkpoint inhibition using the CTLA-4 antibody YH001 and PD-L1 antibody envafolimab combined with or without doxorubicin may improve response rates in patients with advanced or metastatic sarcoma, without undue toxicity.

Methods: This is a multicenter, open label, Phase 1/2 study. The Phase 1 portion includes the

combination of YH001 (either 2 mg/kg or 4 mg/kg i.v. Q3W) and envafolimab (600 mg SQ Q3W) dual immunotherapy in sarcoma patients refractory to or intolerant to standard treatment (i.e., doublet therapy at two YH001 dose levels of > 6 patients each) and YH001 and envafolimab in combination with of doxorubicin (75 mg/m2 Q3W up to six doses) in patients who have not received prior doxorubicin treatment (i.e., triple therapy at two YH001 dose levels of > 6 patients each). Phase 2 cohorts will assess the efficacy of triple therapy in patients with advanced or metastatic LMS or DDLS who are candidates for doxorubicin and have not received doxorubicin or immune checkpoint inhibitor treatment and doublet therapy in patients with advanced or metastatic ASPS and chondrosarcoma who have not received immune checkpoint inhibitor treatment.

Results: As of June 1, 14 patients were enrolled (male:female 8:6, median age = 62 years; all ECOG = 1; 12 White, 1 Asian, 1 other) in the phase 1 portion. Six patients each received YH001 at 2 mg/kg or 4 mg/kg with envafolimab (doublet therapy) resulting in one DLT of grade 3 thyroiditis at the higher YH001 dose. Two patients received triple therapy with YH001 at 2 mg/kg and one patient experienced a DLT of grade 3 fatigue that required doxorubicin dose reduction. The most frequent YH001 related AE was grade 1 or grade 2 pruritis (N=3). Ongoing partial responses have been observed in a patient with angiosarcoma on doublet therapy and in a patient with dedifferentiated chondrosarcoma on triple therapy.

Conclusion: YH001 has been tolerable with envafolimab with or without doxorubicin and evidence of activity has been observed for doublet and triplet therapy.

(P 113) IMMUNOSARC II: A SPANISH SARCOMA GROUP (GEIS) PHASE IB TRIAL OF DOXORUBICIN AND DACARBAZINE PLUS NIVOLUMAB IN FIRST LINE OF ADVANCED LEIOMYOSARCOMA

Javier Martin-Broto, MD, PhD[1]; Roberto Diaz Díaz-Beveridge, PhD, MD[2]; David S. Moura, PhD[3]; Rafael Ramos, MD, PhD[4]; Javier Martinez-Trufero, MD[5]; Irene Carrasco Garcia, MD[6]; Antonio López Pousa, MD[7]; Enrique Gonzalez-Billalabeitia, MD[8]; Antonio Gutierrez, MD, PhD[9]; Josefina Cruz, MD[10]; Ana Sebio, MD, PhD[7]; Ana Herrero, MD[11]; Claudia Valverde, MD[12]; Nadia Hindi, MD, MSc[1], (1) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain, (2) Medical Oncology Department, Hospital Universitario y Politécnico La Fe, Valencia, Spain., Valencia, Comunidad Valenciana, Spain, (3) Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM),, Madrid, Madrid, Spain, (4) Hospital Universitario Son Espases, Palma Mallorca, Islas Baleares, Spain, (5) HU Miguel Servet, Zaragoza, Aragon, Spain, (6) Virgen del Rocio University Hospital, Seville, Andalucia, Spain, (7) Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain., Barcelona, Catalonia, Spain, (8) HU Doce de Octubre, Madrid, Madrid, Spain, (9) HU Son Espases, Palma, Islas Baleares, Spain, (10) HU Canarias, Santa Cruz de Tenerife, Canarias, Spain, (11) Hospital General Villalba, Villalba, Madrid, Spain, (12) Vall D'Hebron University Hospital, Barcelona, Catalonia, Spain

Objective: The immunogenic cell death (ICD) caused by certain chemotherapy agents consists of molecular changes in tumor dying cells that stimulate immunogenicity and enhance antitumor effects. Doxorubicin is a drug with recognized ICD induction capacity, mainly through calreticulin membrane translocation that elicits immunogenic signals as phagocytosis by

dendritic cells. The combination of doxorubicin and pembrolizumab was explored in a phase I/II trial treating patients with metastatic/unresectable sarcomas, achieving Partial Response (PR) in the 22% of patients, stable disease (SD) in the 59%, and progressive disease (PD) in the 19%, with median progression-free survival (mPFS) of 8.1 months, superior to historical controls. We hypothesized that the addition of an anti-PD1 (nivolumab) would increase the antitumor activity of doxorubicin plus dacarbazine, a commonly used upfront polychemotherapy regimen in advanced leiomyosarcoma (LMS) . We present here the phase Ib, cohort 7b of IMMUNOSARC trial.

Methods: Adult patients, with good performance status (ECOG 0-1), naïve of previous anthracycline-containing treatments and with centrally confirmed diagnosis of advanced/metastatic LMS were eligible. Initial dose level 0 (L0) was defined as doxorubicin (DOX) 75 mg/m2/day in 20 min on D1 followed by dacarbazine (DAC) 400 mg/m2/day in 60 min on D1 and 2, plus nivolumab (NIV) 360 mg on D2 after DAC every 21 days with GCSF support. This combo would be given up to 6 courses of 21-day cycles, followed by 1-year NIV maintenance. A -1 dose level (L-1) was defined with the same regimen but with NIV 240 mg. A classic 3+3 phase 1 design was used to determine the recommended phase 2 dose (RP2D) based on dose-limiting toxicities (DLTs), which was the main endpoint, observed during the first 21-day cycle. An expansion of the cohort with RP2D up to 20 evaluable patients was foreseen. Secondary endpoints included overall response rate (ORR), safety profile, translational objectives, among others. A baseline tumoral biopsy and blood sampling baseline and every 6 weeks were mandatory for translational purposes. Levels of expression of HMGB1 were assessed in plasma at week 6 and compared with baseline levels.

Results: Between January 2022 and February 2023, 20 patients (6 Male/14 Female), ECOG 0/1 (15/5), with median age 54 years (range 31-72) were enrolled. All patients were treated with the initial L0 scheme and no DLTs were observed being L0 the RP2D. Grade 3-4 toxicities were neutropenia (20%), anemia (10%), febrile neutropenia, asthenia, alopecia, and GGT increased (5% each). Four patients were not evaluable for efficacy at the moment of the data lock (1 due to uncompliant dosing and in 3 patients the first tumor assessment was not reached yet at the data lock). Of 16 efficacy-evaluable patients, RECIST ORR according to local clinical site assessment was 9 PR (56.3%), 6 SD (37.5%), and 1 PD (6.3%) (2 SD cases showed tumor size reductions >20%). Five patients ended treatment due to progression (4 radiological, 1 clinical) and 15 patients remain under the trial therapy. With a median follow-up of 8 months (2-12), in the 16 evaluable patients, the mPFS was 8.67 months (95% CI 7.96-9.37). In 12 patients, paired blood samples (baseline and week 6) were analyzed for HMGB1 levels. In 10/12 patients HMGB1levels increased at week 6 when compared with baseline. In the univariate analysis, increases in HMGB1levels of at least 11.6% correlated with a better mPFS (8.4 (95% CI 6.1-10.6 vs not reached, p=0.029).

Conclusion: DOX 75 mg/m2/d on D1 followed by DAC 400 mg/m2/d on D1 and 2, plus NIV 360 mg on D2 after DAC Q3W, followed by 1-year NIV maintenance is a feasible and well-tolerated scheme. Clinical activity is encouraging, improving historical efficacy outcomes in first line of advanced LMS, which deserves further testing in phase II trials.

(P 114) PRIMARY ANALYSIS OF EFFICACY AND SAFETY OF LETETRESGENE AUTOLEUCEL (LETE-CEL) AS FIRST-LINE TREATMENT FOR UNRESECTABLE OR METASTATIC SYNOVIAL SARCOMA (SS); SUBSTUDY 1 OF IGNYTE-ESO

Melissa A. Burgess, MD[1]; Jonathan Noujaim, MD[2]; Samuel John, MD[3]; John Charlson, MD[4]; Andrew J.S Furness, MBBS, MRCP, PhD[5]; John B.A.G Haanen, MD, PhD[6]; Jessica Preston, MSc[7]; Stefan Zajic, PhD[8]; Jimson W. D'Souza, MD[9]; Thomas H. Faitg, PhD[10]; Michael J. Nathenson, MD[11]; Sandra P. D'Angelo, MD[12], (1) University of Pittsburgh Medical Center Hillman Cancer Center, Pittsburgh, PA, (2) Hôpital Maisonneuve-Rosemont and McGill University Health Center, Montreal, PQ, Canada, (3) UT Southwestern Medical Center, Dallas, TX, (4) Medical College of Wisconsin, Wauwatosa, WI, (5) The Royal Marsden NHS Foundation Trust, London, England, United Kingdom, (6) Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands, (7) Veramed, London, England, United Kingdom, (8) GSK, Collegeville, PA, PA, (9) GSK, Collegeville, PA, (10) GSK, Philadelphia, PA, (11) Adaptimmune, Philadelphia, PA, (12) Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Lete-cel is an autologous T-cell therapy targeting NY-ESO-1, which is expressed in SS and myxoid round cell liposarcoma (MRCLS). Lete-cel efficacy has been demonstrated in pilot studies for SS and MRCLS for patients (pts) with metastatic or unresectable disease. We explored the feasibility, efficacy, and safety of lete-cel for treatment-naïve pts in the first-line setting for metastatic or unresectable SS or MRCLS.

Methods: IGNYTE-ESO is a master protocol with two substudies (NCT03967223). Substudy 1 (SS1) is an open-label pilot study of lete-cel in treatment-naïve pts with metastatic or unresectable SS or MRCLS. The target enrollment was 10 pts. Key eligibility criteria were: age ≥10 years; HLA-A*02:01, A*02:05, or A*02:06; NY-ESO-1+ (≥30% of cells 2+/3+); optional bridging therapy consisting of 2 cycles of doxorubicin (dox) between apheresis and lymphodepletion (LD); and measurable disease. Pts received fludarabine (range: 60–120 mg/m^2) and cyclophosphamide (range: 1800–3600 mg/m^2) for LD, and a transduced T-cell dose between 1 to 15x10^9. LD dose reduction was allowed for age ≥60 years and renal impairment. Response was assessed at weeks 6, 12, 18, 24, then every 3 months (mo) until disease progression, death or withdrawal. The primary efficacy endpoint was investigator-assessed overall response rate (ORR) by RECIST v1.1. Secondary endpoints included disease control rate (DCR), time to response (TTR), duration of response (DOR), progression-free survival (PFS), and safety. To characterize the cellular pharmacokinetics of lete-cel we report the AUC from time 0 to day 28, and Cmax.

Results: Data cut: January 27, 2023. The primary analysis was conducted when all enrolled pts had received T-cell infusion and completed ≥2 post-baseline disease assessments or withdrawn. From January 2020 to May 2022, 52 pts screened for SS1, 7 pts enrolled, and 5 pts received LD and T-cell infusion (median T-cell dose 5.4x10^9). Due to slow accrual, study enrollment was terminated prior to reaching target enrollment. Two pts were not eligible for LD and T-cell infusion (developed a 2nd malignancy [1 pt] and received >2 cycles of dox for bridging therapy [1 pt]). All enrolled pts had SS. All 5 treated pts were metastatic at screening. One pt had a history of neoadj/adj chemo, and 1 pt received bridging chemo. Median time from date of locally advanced or metastatic disease to leukapheresis was 2.4 (range: 1.6–5.9) mo. Median time from leukapheresis to T-cell infusion was 2.3 (range: 2.1–11.3) mo. Median follow-up for PFS was 7.0 mo. The ORR was 80% (95% CI: 28.4–99.5%), with 1 CR and 3 PR. 1 pt had SD as best response, yielding a DCR of 80% (95% CI: 28.4–99.5%). Median TTR was 1.2 (range: 1.1–2.6) mo for the 4 pts with CR/PR. The median DOR and PFS were not mature, as

the 4 responders were ongoing at the time of this analysis. The pt with SD as best response developed clinical disease progression, grade 5 hemoptysis from pulmonary metastases, and died from their disease. All pts had ≥1 treatment emergent adverse event (TEAE), 3 pts had ≥1 serious TEAE. The most common TEAEs were cytokine release syndrome (100%; grade 1 [3], grade 2 [1], grade 3 [1]), ALT increase (60%), fatigue (60%), and neutropenia (60%). 2 pts developed GvHD predominated by rash. Geometric means were 1,911,782.96 (%CV: 53.709) copies per µg gDNA times days for AUC(0–28d), and 119,701.64 (%CV: 68.267) copies per µg gDNA for Cmax. The 4 pts who responded to therapy continue to have detectable T-cell persistence during follow-up.

Conclusion: This study highlights the challenge to enroll advanced/metastatic treatment-naïve rare sarcoma pts in a cell therapy trial, with 7 pts enrolled over ~2.5 years. Encouraging efficacy was seen in a small population of treatment-naïve pts in the advanced/metastatic setting with 80% ORR, with all responses ongoing at the time of this analysis. The TEAEs for SS1 are consistent with the known safety profile of lete-cel. All responders have prolonged T cell persistence.

Table 1: IGNYTE-ESO Substudy 1 ORR, DCR, TTR, DOR, and PFS

Investigator assessed endpoints	**Lete-cel (N=5)**
ORR rate (95% CI)	80% (28.4%, 99.5%) (1 CR, 3 PR)
DCR rate (95% CI)	80% (28.4%, 99.5%) (1 CR, 3 PR)
TTR (months) range / median	1.1–2.6 / 1.17
DOR (months) median (95% CI) [+ indicates ongoing]	Median not calculable, not mature 4 censored: 3.0+, 4.4+, 7.5+, 14.5+ Median follow up: 5.9 (3.0, 14.5)
PFS (months) median (95% CI) [+ indicates ongoing]	Median not calculable, not mature 1 progression: 1.7 4 censored: 5.6+, 5.6+, 8.5+, 15.6+ Median follow-up: 7.0 (5.6, 15.6)

Figure 1. IGNYTE-ESO Substudy 1 Spider Plot of Investigator-Assessed Percent Change from Baseline in Target Lesion Diameter per RECIST v 1.1

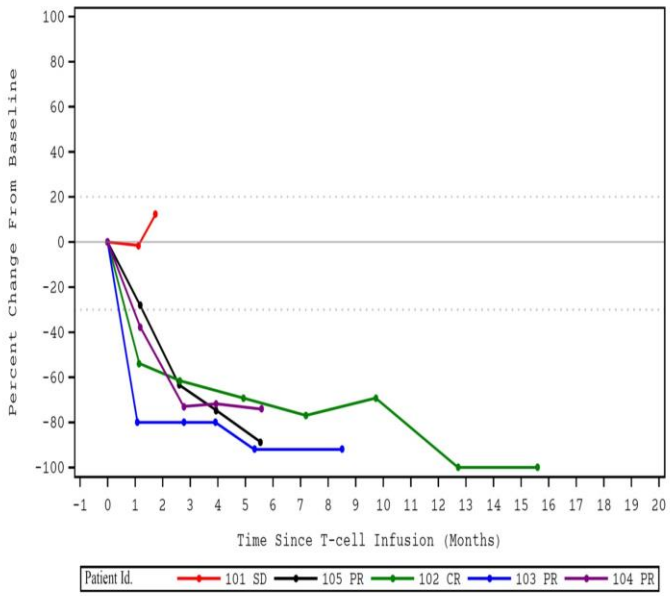
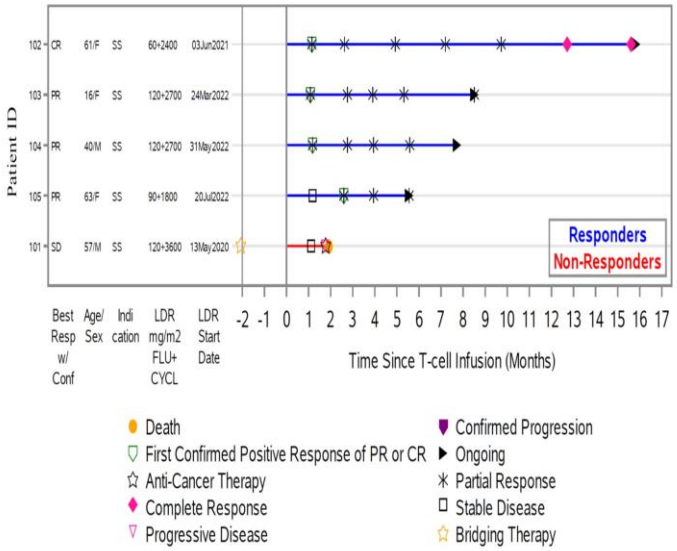


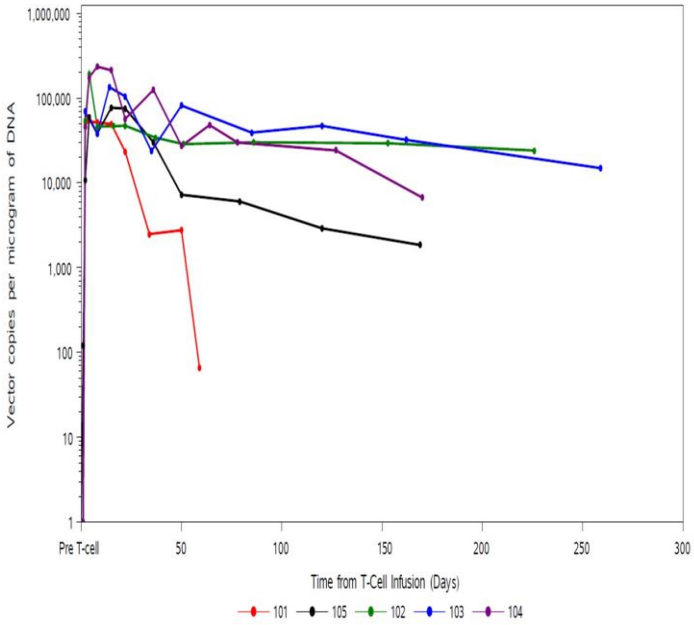
Figure 2. IGNYTE-ESO Substudy 1 Swimmers Plot of Duration on Interventional Phase



Interventional Phase is defined as the time between Lymphodepletion and the time at which the patient either progresses, dies or withdraws from treatment.

Conf=confirmation; CR=complete response; CYCL=cyclophosphamide; F=female; FLU=fludarabine; LDR=lymphodepletion regimen; M=male; PR=partial response; SD=stable disease; SS=synovial sarcoma; w/=with

Figure 3. IGNYTE-ESO Substudy 1 Plot of T-cell Persistence by Concentration over Time



(P 115) EFFICACY AND CORREALTIVE ANALYSIS OF USING NEOADJUVANT INTRATUMORAL HIGH DOSE TALIMOGENE LAHERPAREPVEC (TVEC) WITH CONCURRENT RADIATION IN HIGH-RISK EXTREMITY AND TRUNK SOFT-TISSUE SARCOMAS

Varun Monga, MBBS[1]; John M. Rieth, MD[2]; Benjamin J. Miller, MD[2]; Bryan Allen, MD, PhD[2]; Margaret Kozak, MD[3]; Carryn Anderson, MD[2]; Munir Tanas, MD[2]; Jonathan Davick, MD[2]; Laura Pietrok, PhD[4]; Ann Miller, PhD[5]; Zeb Zacharias, PhD[2]; Jon Houtman, PhD[2]; Steven Varga, PhD[6]; Sarah Mott, MS[7]; Mohammed Milhem, MBBS[8], (1) UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, (2) University of Iowa, Iowa City, IA, (3) UT Southwestern, Dallas, TX, (4) Cerberus Therapeutics, Cambridge, MA, (5) St Jude's Children's Hospital, Memphis, TN, (6) St. Jude Children's Research Hospital, Memphis, TN, (7) Holden Comprehensive Cancer Center, Iowa City, IA, (8) University of Iowa, Holden Comprehensive Cancer Center, Iowa City, IA

Objective: Intratumoral oncolytic virus Talimogene Laherparepvec (TVEC) given concurrently with standard of care neoadjuvant external beam radiation (EBRT) has previously been shown to be safe for treatment of localized high risk soft tissue sarcoma (STS). TVEC probably enhances anti-tumor immune responses through both direct infection and GM-CSF. At a given time 4mL of 108 PFU/mL is the maximum volume of TVEC approved by FDA for injection of melanoma lesion. Given the large size of primary sarcomas, in this study we used 8mL of 108 PFU/mL of TVEC injection after initial seroconverting dose to achieve better pathological responses in this study. We report the efficacy and immune correlates of this single center phase IB/II study of TVEC 8 mL of 108 PFU/mL dosing, in addition to EBRT as neoadjuvant treatment for high-risk extremity and trunk STS.

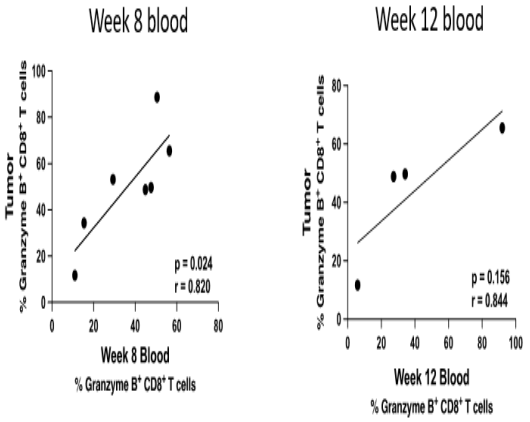
Methods: A total of 8 patients with locally advanced high risk (size > 5 cm and Grade >/= 2) STS of the extremities and trunk were treated with intratumoral TVEC in addition to standard-of-care EBRT followed by surgical resection. EBRT doses of 50 Gy were delivered over 25 fractions, in addition to weekly TVEC dosed at 8 mL of 108 PFU/mL after the first seroconverting dose of 4mL of 106 PFU/mL. Resection of the tumor was performed 4-6 weeks after completion of EBRT. Tumor responses were assessed as per RECIST 1.1 from baseline to pre-operative imaging. Fresh tumor biopsies were obtained at the time of diagnosis prior to starting treatment and resected tumor tissue was obtained at the time of primary tumor resection. Peripheral blood specimens were obtained at baseline, at the end of radiation, and pre-operatively. Using flow cytometry immune cell studies were performed in disaggregated tissue specimens as well as peripheral blood samples. Trial accrual was stopped after 8 patients. Comparator tumor samples from patients undergoing only standard EBRT were taken as part of a separate study.

Results: Of the 6 patients treated in Phase 1, 0 out of 6 experienced a dose limiting toxicity. Adverse events (AEs) attributed to TVEC included grade 3 fatigue (12.5%) and dermatitis (12.5%). Treatment related grade 1-2 AEs included nausea (37.5%), chills (75.0%), anorexia (25.0%), fever (62.5%). Of the eight patients, four patient tumor specimens (50%) achieved >90% tumor necrosis. Three (37.5%) patient tumors achieved a partial response (undifferentiated sarcoma, dedifferentiated liposarcoma and myxoid round cell liposarcoma) and 5 patients had stable disease. Granzyme B producing CD8+ T cells in the blood were noted to increase between week 1 and week 8 ($p< 0.05$) of treatment. A strong positive correlation was noted between granzyme B+ CD8+ T cells in the blood at 8 and 12 weeks of treatment vis a vis in the resected tumor. CD8+ T cells were noted to be higher in TVEC+ EBRT treated tumors relative to EBRT alone treated comparator tumor samples ($p< 0.05$). In addition, an increase in CD8+ T cells was evidenced between the pre-treatment biopsy and the post treatment resected tumor among TVEC+ EBRT treated ($p< 0.01$). The CD4+ T cell population was significantly reduced in the surrounding tissue compared to the tumor ($p< 0.05$). Cytokine analysis in the blood revealed that higher pre-treatment Epidermal Growth Factor was associated with higher tumor necrosis ($p< 0.05$).

Conclusion: Neoadjuvant intratumoral TVEC at 8 mL of 108 PFU/mL dosing with concurrent EBRT increased pathological necrosis rate relative to EBRT alone for locally advanced STS. Intratumoral TVEC treatment in addition to EBRT increases cytotoxic CD8+ T cells in STS compared with EBRT alone treated and pre-treatment biopsy sarcoma tissue. Overall, no new safety signals were observed. Higher doses of TVEC with EBRT should be explored in STS.

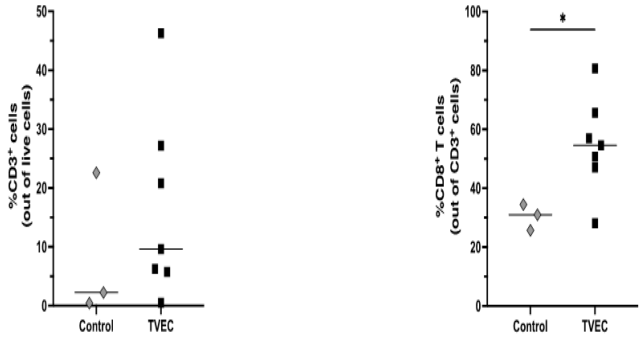
TVEC + EBRT treated post-resection tumors vs blood

Comparing CD8 T cells that produce Granzyme B



Strong Pearson r values from weeks 8 and 12 showing strong positive correlation between Granzyme B+ CD8+ T cells in the blood and in the tumor. Statistics: Simple linear regression

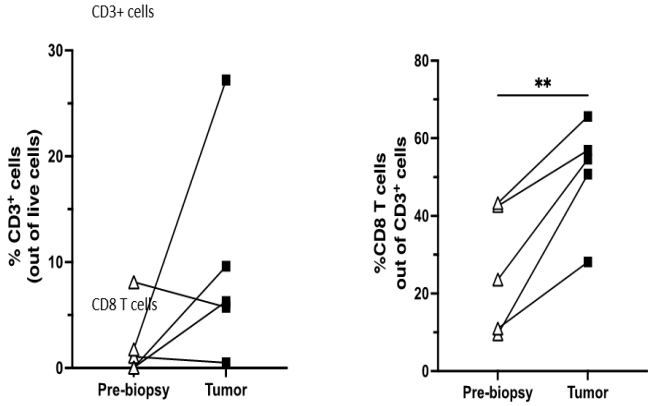
EBRT alone vs TVEC + EBRT treated resected sarcoma tumors



*p < 0.05 Statistics: unpaired t test

Comparing the frequency of cells between control and TVEC treated tumor there is significant increase in CD8+ T cells in TVEC + EBRT vs EBRT alone control primary sarcoma tumors

Pre-treatment biopsy samples vs TVEC + EBRT treated resected tumors



**p < 0.01 Statistics: paired t test

Examining the difference in frequencies of cells between pre-core biopsy and post-resection tumor in TVEC treated patients. The CD8 + T cells were noted to be higher in TVEC + EBRT treated primary tumors vs corresponding pre-treatment biopsy specimens

(P 116) UPDATE ON SAINT: RESULTS OF 100 PATIENTS TREATED WITH IPILIMUMAB (I), NIVOLUMAB (N) AND TRABECTEDIN (T) AS FIRST LINE THERAPY FOR ADVANCED SOFT TISSUE SARCOMA [NCT03138161]

Nadezhda Omelchenko, MD[1]; Samantha Jeffrey, BS Pre-Med[1]; Angel L. Orosco-Ttamina, MD[1]; Elan Younesi, BS Pre-Med[1]; Vivek Gujrathi, BS Pre-Med[1]; Neal S. Chawla, MD[1]; Don Brigham, PhD[1]; Victoria Chua, MD, CLS[1]; Doris Quon, MD, PhD[1]; Ania Moradhkani, NP[1]; Sant P. Chawla, MD, FRACP[1]; Erlinda M. Gordon, MD[2]; Stefan Makrievski, MD[1], (1) Sarcoma Oncology Center, Santa Monica, CA, (2) Sarcoma Oncology Research Center, Santa Monica, CA

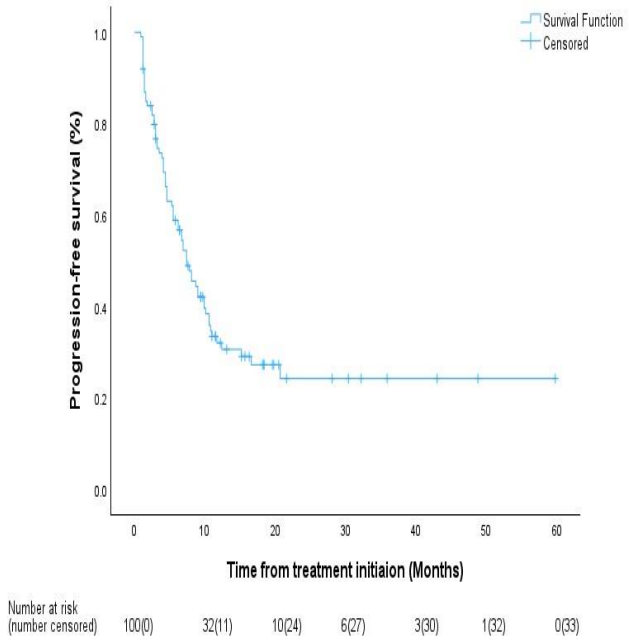
Objective: Advanced soft tissue sarcoma is most often associated with a fatal outcome. Recently, we reported on the safety and efficacy of the SAINT protocol in 79 previously untreated patients with advanced soft tissue sarcoma. This report is an update of SAINT on 100 patients treated with SAINT earlier in the course of the disease.
Objectives: Primary: Evaluate best response rate by RECIST v1.1; Secondary: Assess progression-free survival (PFS) at 6 months and overall survival.

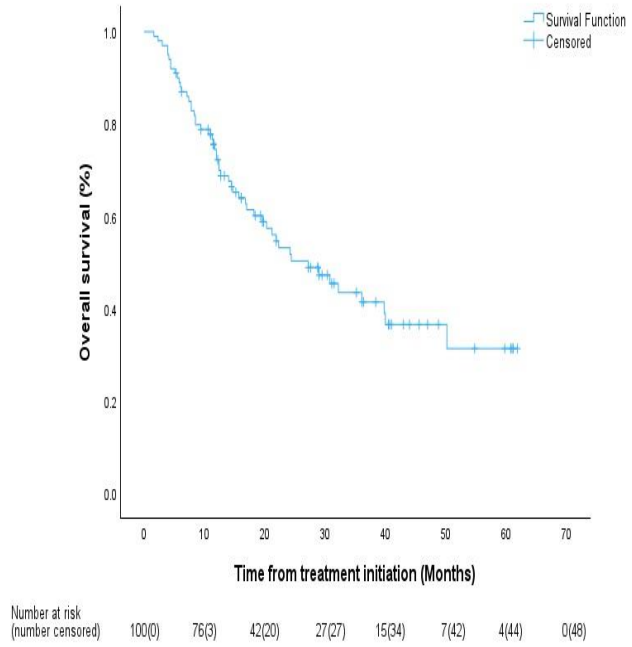
Methods: Eligible patients for this Phase 2 study are males or females ≥ 18 years of age with locally advanced unresectable or metastatic soft tissue sarcoma, previously untreated, with measurable disease by RECIST v1.1. Treatment protocol: (I) 1 mg/kg i.v. q 12 wks, (N) 3 mg/kg i.v. q 2 wks, (T) 1.2 mg/m2 CIV q 3 wks.

Results: Results: Efficacy: One hundred patients were evaluated for efficacy. These subjects completed at least one treatment cycle and had a follow-up CT scan. The best responses were 7CR, 13PR, 64SD and 16PD with 20% ORR, 84% DCR. PFS rate at 6 months was 55%;

Median PFS was 7.4 (95% CI: 5.4-9.4) months; Median OS was 27.2 (95% CI: 17.7-36.7) months. The best responders (CR and PR) were patients with LMS, UPS, LPS, synovial sarcoma, myxofibrosarcoma, endometrial stromal sarcoma, clear cell sarcoma, DSRCT, and chondrosarcoma. Notably, out of the 100 patients who participated in the study between 2017 and 2022, nineteen (19%) patients are still alive as of June 1, 2023, the data cut-off date. Safety: Grade 3/4 TRAEs related to Trabectedin include both hematologic and non-hematologic toxicities: fatigue, nausea, vomiting, fever, exhaustion, dehydration, asthenia, cellulitis of port, anemia, neutropenia, thrombocytopenia, transaminitis, elevated CK. There were no hematologic toxicities related to Nivolumab or Ipilimumab. Grade 3/4 TRAEs include decreased TSH, increased T4, increased TSH, transaminitis, hyponatremia, dehydration, pruritus, and psoriasis.

Conclusion: Conclusions: Taken together we have confirmed that (1) The SAINT regimen is a safe and effective first line therapy for advanced soft tissue sarcoma, and (2) Randomized studies are warranted to confirm whether the SAINT regimen is superior to standard first line therapy for advanced soft tissue sarcoma.





(P 117) INTERIM ANALYSIS OF PHASE 2 STUDY: A METRONOMIC REGIMEN USING GEMCITABINE, DOXORUBICIN, DOCETAXEL AND NIVOLUMAB FOR ADVANCED LEIOMYOSARCOMA (NCT04535713)

Angel L. Orosco-Ttamina, MD[1]; Nadezhda Omelchenko, MD[1]; Stefan Makrievski, MD[1]; Guilherme Leão De Melo, MD[1]; Neal S. Chawla, MD[1]; Don Brigham, PhD[1]; Amir Ahari, MD[1]; Victoria Chua, MD, CLS[1]; Doris Quon, MD, PhD[1]; Ania Moradhkani, NP[1]; Steven G. Wong, MD[1]; Mahesh Seetharam, MD FACP[2]; Sant P. Chawla, MD, FRACP[1]; Erlinda M. Gordon, MD[3], (1) Sarcoma Oncology Center, Santa Monica, CA, (2) Mayo Clinic, Phoenix, AZ, (3) Sarcoma Oncology Research Center, Santa Monica, CA

Objective: Metronomic dosing of gemcitabine, doxorubicin and docetaxel causes less severe side effects than standard chemotherapy for advanced leiomyosarcoma. Hypothesis: The addition of nivolumab to this regimen will have synergistic effects and improve treatment outcomes. Primary objective: To assess progression-free survival (PFS); Secondary objectives: (1) To evaluate best overall response, (2) PFS rate at 6 and 9 months, (3) Overall survival (OS) rate at 6, 12 months, and (4) Incidence of treatment-related adverse events (TRAEs).

Methods: Inclusion criteria: Previously treated male and female subjects, > 18 years of age, pathologically confirmed diagnosis of locally advanced, unresectable, or metastatic leiomyosarcoma, measurable disease by RECIST v1.1, and acceptable hematologic and organ functions. Exclusion Criteria: History of autoimmune disorder. Treatment schedule: Metronomic doses of gemcitabine (600 mg/m2 max:1000 mg), doxorubicin (18 mg/m2; max: 32 mg), docetaxel (25 mg/m2; max:42 mg) on Day 1 and Day 8, and nivolumab (240 mg) on Day 1 only. Repeat treatment cycles may be given every three weeks if the toxicity grade is < 1.

Results: Efficacy (n=17). This population completed at least one treatment cycle and had a follow-up CT or MRI scan at week 6. Confirmed best overall response = 4 PR, 11 SD, 2 PD. The disease control rate (CR+PR+SD) was 88.2%. Median PFS was 6.3 (95% CI: 2.837-7.363) months; 6-month PFS rate 53%. Kaplan Meir curve for PFS is shown in Figure. Median OS for ITT population (n=18) was 15.5 (95% CI: 5.48-25.12) months, with 6-month OS 94%. As of data cut-off date, median OS for mITT population has not yet been reached. Safety (n=18): Grade 3/4 TRAEs include thrombocytopenia (n=9), neutropenia (n=8), white blood cell count decreased (n=7), anemia (n=4), nausea (n=3), anorexia (n=1), diarrhea (n=1), fatigue (n=1), rectal bleeding (n=1), transaminitis (n=1). There were no unexpected adverse events reported.

Conclusion: Taken together, the data suggests that nivolumab plus metronomic doses of gemcitabine, doxorubicin, and docetaxel (1) may have synergistic activity, and (2) is a promising second/third line combination regimen for advanced leiomyosarcoma with manageable toxicity.

(P 118) TIM-3 QUALIFIES AS A POTENTIAL IMMUNOTHERAPEUTIC TARGET IN SPECIFIC SUBSETS OF PATIENTS WITH HIGH-RISK SOFT TISSUE SARCOMAS (HR-STS)

Luc M. Berclaz, MD[1]; Annelore Altendorf-Hofmann, MD[2]; Lars H. Lindner, MD[3]; Anton Burkhard-Meier, MD[1]; Dorit Di Gioia, MD[3]; Hans Roland Dürr, MD[4]; Alexander Klein, MD[4]; Markus Albertsmeier, MD[5]; Nina-Sophie Schmidt-Hegemann, MD[6]; Frederick Klauschen, MD[7]; Thomas Knösel, MD[7], (1) Department of Internal Medicine III, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (2) Department of General, Visceral and Vascular Surgery, Friedrich-Schiller University Jena, Germany, Jena, Thuringen, Germany, (3) Department of Medicine III, LMU University Hospital, LMU Munich, Munich, Bayern, Germany, (4) Orthopaedic Oncology, Department of Orthopaedics and Trauma Surgery, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (5) Department of General, Visceral and Transplantation Surgery, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (6) Department of Radiation Oncology, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (7) Institute of Pathology, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany

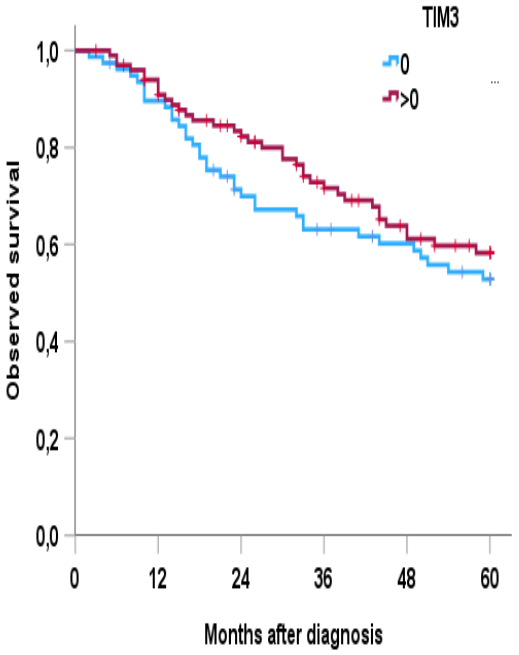
Objective: The expression of T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), an immune checkpoint receptor on T cells, has been associated with dismal outcomes and advanced tumor stages in various solid tumors. The blockade of TIM-3 is currently under examination in several clinical trials. This study examines TIM-3 expression in high-risk soft tissue sarcomas (HR-STS).

Methods: Tumor cell expression of TIM-3 on protein level was analyzed in pre-treatment biopsies of patients with HR-STS. TIM-3 expression was correlated with clinicopathological parameters including tumor-infiltrating lymphocyte (TIL) counts, programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PDL-1) expression in patients with HR-STS. Survival dependent on the expression of TIM-3 was analyzed.

Results: TIM-3 expression was observed in 101 (56%) out of 179 pre-treatment biopsies of patients with HR-STS. TIM-3 expression was significantly more often observed in undifferentiated pleomorphic sarcomas (UPS) compared to other histological subtypes (p <

0.001), high TIL counts ($p < 0.001$), high PD-1 ($p < 0.001$) and PD-L1 expression ($p < 0.001$). TIM-3 expression did not have a prognostic impact on survival in patients with HR-STS.

Conclusion: This is the first study to demonstrate a significant tumor cell expression of TIM-3 in specific subsets of patients with HR-STS. TIM-3 qualifies as a potential immunotherapeutic target in HR-STS.



Factor	Strata	Total	TIM3 >0		p-value
		n	n	%	
Sex	Male	92	59	64	**0.036**
	Female	87	42	48	
Age at initial diagnosis (years)	<55	92	43	47	**0.010**
	≥55	87	58	67	
Histological subtype	UPS	59	44	75	**<0.001**
	Liposarcoma	31	11	35	
	Leiomyosarcoma	40	26	65	
	Other	49	20	41	
Tumor Location	Extremities	71	47	66	**0.045**
	Non-extremities	108	54	50	
FNCLCC Grade	Intermediate (G2)	89	46	52	0.229
	High (G3)	90	55	61	
Surgical margins	R0	69	48	70	**0.011**
	R1	83	41	49	
	R2	14	4	29	
	No resection	13	8	62	
Chemotherapy	Yes	134	80	60	0.164
	No	45	21	47	
Radiotherapy	Yes	30	16	53	0.535
	No	106	48	45	
	Missing	43			
Regional Hyperthermia (RHT)	Yes	139	86	62	**0.007**
	No	40	15	38	
TIL counts (cells/50HPF)	0-5	108	46	43	**<0.001**
	≥6	70	54	77	
	Missing	1			
PD-1 expression	0	61	18	30	**<0.001**
	≥0	77	46	60	
	Missing	41			
PD-L1 expression	0	139	66	47	**<0.001**
	≥0	34	31	91	

(P 119) **EX VIVO EXPANDED TUMOR INFILTRATING LYMPHOCYTES FOR SARCOMA TREATMENT**

Harjeet Singh, PhD; Simon Olivares, BS; Fiorela Hernandez, MD; Wendong Zhang, BS; Nancy Gordon, MD; Vidya Gopalakrishnan, PhD; Jonathan Gill, MD; Richard Gorlick, MD, The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: Sarcomas are rare cancers that develop in the bone and soft tissues, with osteosarcoma being the most common primary malignant bone tumor, affecting both children and young adults. Despite advances in treatment, metastatic disease, particularly to the lungs, remains the most prominent cause of osteosarcoma-related deaths. Adoptive cell therapy using tumor-infiltrating lymphocytes (TILs) has shown durable clinical responses in metastatic melanoma patients and is being investigated for other solid tumors. However, TIL isolation and expansion in osteosarcoma and soft-tissue sarcomas is limited. Although TIL generation from sarcomas has been successful, the proportion of patients that can reliably generate a product of clinically meaningful number is lower (30%) than reported in melanoma (95%) along with inconsistently low tumor-specificities. Rapid expansion protocol (REP) the known method of expanding TILs has shown low success rate of TIL expansion (50%) from liposarcoma samples by our group. Discontinuation of anti-41BB antibody commercially along with high cost and variability of generating feeder cell layer for REP begs the need to develop a platform for expansion of TILs to clinical meaningful numbers. Artificial antigen presenting cells (AaPC) developed from K562 cells, expressing desired T-cell costimulatory molecules, are an appealing “off-the-shelf” feeder cells for human application. We have previously successfully utilized K562-AaPC to expand genetically modified T cells from peripheral blood for clinical trials. These K562 AaPC have also shown expansion of TILs comparable to REP from melanoma patients.

Methods: In the current study we used K562 AaPC expressing CD64, CD86, CD137L along with IL15/IL15Rα fusion protein (membrane-bound IL-15; mIL15) to expand TILs from sarcoma patients. Enzymatically dissociated single cell suspension from tumor tissue was co-cultured with irradiated OKT3 loaded K562 AaPC along with addition of exogenous cytokines (IL-2, and IL-21) in a 7-day stimulation cycle, and ex vivo expanded TILs were phenotypically and functionally characterized.

Results: Our data shows that K562 AaPCs have a 100% success rate of TIL expansion from sarcoma samples (n=5; Osteosarcoma=3, high grade undifferentiated sarcoma=1, sarcoma of left anterior shoulder=1) yielding (mean) 1.6e14 cells in (mean) 43.5 days of co-culture. K562 AaPC ex vivo expanded cells were predominantly CD8 (mean CD4/CD8, 0.09) and the platform generated a polyclonal TCR Vβ repertoire of the TIL product where all 24 Vβ families tested were preserved in the product. TILs generated were activated and capable of degranulation (mean CD107a, 35%) and anti-tumor response (mean IFN-g, 78.4%) upon stimulation. This was evident by targeting and lysis of autologous PDX tumors by TILs in a standard 4h chromium release cytotoxicity assay. TILs expressed memory cell markers (CCR7, CD62L, CD45RO) as well as determinants of an effector-cell phenotype. More than half of the TILs (mean, 88.7%) expressed CCR7, and half (mean, 48%) of all TILs expressed markers for central memory (Tcm, CD45RO+CD62L+). Co-expression of CCR7 and CD62L distinguishes a putative subset of memory T cells that have potential to home to lymph nodes.

Conclusion: These data support the hypothesis that K562 AaPCs are an ideal and superior ex vivo expansion platform for generation of activated TILs from sarcoma patients with memory phenotype which have implication for improved efficacy and long-term persistence after

adoptive transfer.

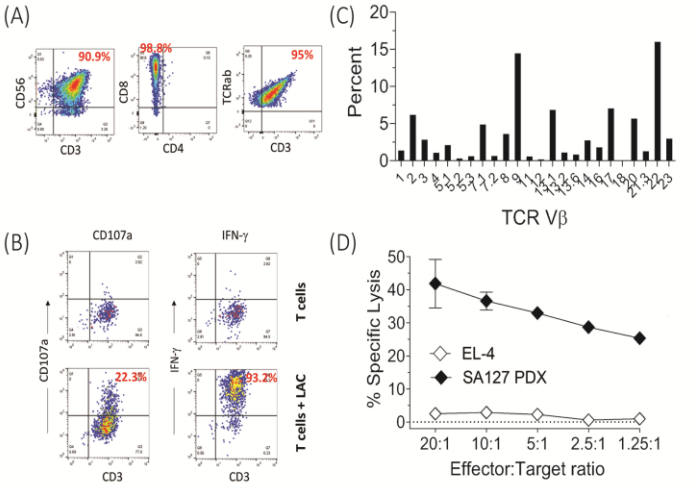


Figure: Characterization of TILs isolated from sarcoma patient SA127. (A) Phenotypic characterization of TILs isolated from osteosarcoma patient tumor tissue at the end of co-culture is shown. Enzyme digested tumor lysate was co-cultured with with K562-derived AaPC every 7-days in a stimulation cycle; at the end of the stimulation cycle T-cells were enumerated and evaluated by flow cytometry. Viable cells were gated for CD3+ T cells and analyzed for the presence of CD56, CD4, CD8 and TCRαβ using flow cytometry. TILs isolated are mostly of the CD8-subtype. (B) Functional potential (CD107a, IFN-γ) of *ex vivo* expanded TILs was evaluated by their ability to respond to extracellular stimulation—Leukocyte Activation Cocktail (LAC, BD Biosciences) (containing PMA and Ionomycin). (C) Quantitative determination of TCR Vβ repertoire of *ex vivo* expanded TILs was performed using flow cytometry (IOTest Beta Mark Kit, Beckman Coulter). (D) Killing of matched osteosarcoma patient tumor cells by *ex vivo* expanded TILs in a standard 4-hr chromium release assay. Xenogeneic mouse cell line EL-4 was used as control to depict background lysis.

(P 120) **THE FEASIBILITY OF CD47-BASED IMMUNOTHERAPY FOR OSTEOSARCOMA**

YUNMI KO, Ph. D.[1]; JUN AH LEE, MD, PhD[2], (1) National Cancer Center, Goyang-si, Kyonggi-do, Republic of Korea, (2) National Cancer Center, Goyang, Kyonggi-do, Republic of Korea

Objective: Macrophages account for the majority of immune cells infiltrated in sarcoma tissues. CD47 is expressed on many cancer cells and acts as a macrophage immune checkpoint by interacting with SIRPα of the macrophage surface. In this study, we evaluated the feasibility of CD47 as a potential candidate for immunotherapy of osteosarcoma.

Methods: CD47 immunohistochemical staining was performed in 24 biopsy samples obtained from patients younger than 20 years, and the relationships between CD47 expression and clinicopathologic characteristics were evaluated. For KHOS/NP and MG-63 osteosarcoma cells, CD47 protein expression was analyzed using western blotting. From fresh peripheral blood obtained from healthy adult volunteers, monocytes were isolated using human CD14 magnetic microbeads, and cultured with macrophage colony-stimulating factor to differentiate into macrophages. KHOS/NP and MG-63 osteosarcoma cells were labeled with CFSE dyes and pre-treated with CD47 antibody (B6H12) or isotype control and then co-cultured with differentiated macrophages. The efficacy of the CD47 antibody on the phagocytic activity of macrophages was evaluated using flow cytometry.

Results: Of the 24 biopsy samples of osteosarcoma, CD47 protein was expressed in 5 samples. Positive staining for CD47 protein was related to the presence of metastasis at

diagnosis (P=0.04). Patients with tumors stained positive for CD47 protein tended to be older than those with tumors stained negative (16.5□6.2 years vs. 15.7□3.0 years, P=0.07). However, sex, tumor size, and histologic response to preoperative chemotherapy were not associated with CD47 protein expression. Western blot showed that CD47 protein is expressed in both KHOS/NP and MG-63 osteosarcoma cells, with a slightly higher expression level in KHOS/NP cells. CD47 antibody pre-treatment did not influence the proliferation and apoptosis of two osteosarcoma cells. Peripheral blood-derived CD14-positive monocytes were effectively differentiated into macrophages. The phagocytic activity of differentiated macrophages on KHOS/NP osteosarcoma cells was significantly higher in cells pretreated with CD47 antibody, compared to the control. In contrast, the phagocytic activity on MG-63 osteosarcoma cells tended to increase, but there was no significant difference.

Conclusion: Our study results suggest the possibility of CD47 as a new immunotherapeutic target of osteosarcoma. To increase the anti-osteosarcoma efficacy of the CD47 antibody, further studies are necessary to couple the macrophage phagocytosis and effector T cell function.

(P 121) IMPROVING THE EFFICACY OF GD2 CHIMERIC ANTIGEN RECEPTOR T-CELLS USING HISTONE DEACETYLASE INHIBITORS

Meghan Shekar, MD[1]; Lauren Scherer, MD[1]; Candise Tat, MPH[2]; Stacey Van Pelt, n/a[3]; Rebecca L. Murdaugh, PhD[3]; Angela Major, n/a[4]; John Hicks, MD, PhD, DDS[4]; Jamie Anastas, PhD[3]; Cliona Rooney, PhD[2]; Bilal Omer, MD[5], (1) Texas Children's Hospital / Baylor College of Medicine, Houston, TX, (2) Texas Children's Hospital / Baylor College of Medicine Center for Cell and Gene Therapy, Houston, TX, (3) Baylor College of Medicine, Houston, TX, (4) Baylor College of Medicine / Texas Children's Hospital, Houston, TX, (5) Texas Children's Hospital / Baylor College of Medicine, Center for Cell and Gene Therapy, Houston, TX

Objective: Chimeric Antigen Receptor (CAR) T-cell treatment has transformed the landscape of treatment for relapsed / refractory B-lineage hematologic malignancies. Treatment of solid tumors with CAR T-cells is more challenging, in part due to heterogeneous expression of CAR antigens on most solid tumors. Neuroblastoma has uniformly high GD2 expression and has been successfully targeted with GD2.CAR T-cells at our and other centers, but responses for osteosarcoma and other solid tumors with heterogeneous GD2 expression have been mixed. This lack of high and uniform GD2 expression is likely a major factor limiting the success of GD2.CAR T-cells for osteosarcoma and other sarcomas.

HDAC inhibitors (HDACi), an epigenetic modifier drug class, are known to increase GD2 expression on tumors in vitro. We hypothesize that these drugs can be used in combination with GD2.CAR T-cells to synergistically improve tumor killing, both due to increased GD2 expression and due to the anti-tumor effect of HDACi themselves. Limited data exists on the effect of HDACi on T-cell proliferation and function. We wanted to determine whether any HDACi could (i) boost GD2 expression while (ii) maintaining CAR T-cell function.

Methods: In an initial step we analyzed patient tumor tissue samples by immunohistochemical staining for GD2. To investigate the effects of HDACi on CAR T-cells, we incubated GD2.CAR T-cells with clinically relevant concentrations of different HDACi and measured their proliferation in vitro. We also evaluated the cytotoxicity of GD2.CAR T-cells against osteosarcoma cells, comparing cells pre-treated with an HDACi versus cells without pre-

treatment. In vivo, we established subcutaneous osteosarcoma tumors in NSG mice, then treated them with varying doses of oral HDACi with and without GD2.CAR T-cells to measure tumor GD2 expression and tumor response.

Results: We analyzed 95 patient tumor tissue samples and found GD2 expression to be highly variable in osteosarcoma and other pediatric solid tumors, both by intensity and by proportion of positive cells. (Table 1) Next, we confirmed that HDACi can increase GD2 expression on osteosarcoma cell lines. (Figure 1) We determined that survival and expansion of GD2.CAR T-cells in vitro are limited by HDACi in a concentration-dependent manner with the exception of vorinostat. CAR T-cell expansion decreased by 80% or more by day 21 in 3 of 4 tested HDACi, whereas vorinostat only reduced growth by 20% at this same timepoint. In a xenograft model of osteosarcoma, the addition of vorinostat to a subtherapeutic dose of GD2.CAR T-cells resulted in a moderate decrease in tumor growth (mean tumor volume of 292 mm3 at day 29 compared to volume of 895 mm3, $p < 0.05$) when compared to CAR T-cells alone. (Figure 2) GD2.CAR T-cells persisted and expanded in vivo despite simultaneous and continued oral HDACi administration.

Conclusion: We have shown GD2 expression to be variable and heterogeneous in many solid tumors, thus the ability to overcome this could have wide applications. HDAC inhibitors upregulate GD2 expression on tumor cells in vitro and in vivo. Our preclinical studies suggest vorinostat may be an ideal candidate to use in combination with GD2.CAR T-cells in vivo due to an absence of negative effects on CAR T-cells.

A.

Diagnosis	Number of Patients	GD2 +	GD2 Grading			
			1 (1-25%)	2 (26-50%)	3 (51-75%)	4 (76-100%)
Osteosarcoma	29	69% (20)	3	4	5	8
Rhabdomyosarcoma	13	46% (6)		1	1	4
Ewing Sarcoma	14	50% (7)			2	5
Uveal Melanoma	6	17% (1)				1
Soft Tissue Sarcomas	6	83% (5)	1		2	2
Neuroblastoma	26	96% (25)	1	8	7	9
Retinoblastoma	1	100% (1)			1	
TOTAL	95	65				

B.

Diagnosis	Number of Patients	GD2 +	GD2 Intensity		
			1+	2+	3+
Osteosarcoma	29	69% (20)	2	8	10
Rhabdomyosarcoma	13	46% (6)	1	5	
Ewing Sarcoma	14	50% (7)	3	3	1
Uveal Melanoma	6	17% (1)	1		
Soft Tissue Sarcomas	6	83% (5)	1	3	1
Neuroblastoma	26	96% (25)	3	11	11
Retinoblastoma	1	100% (1)		1	
TOTAL	95	65			

Table 1. Immunohistochemistry results obtained by staining 95 patient tumor tissue samples with murine anti-GD2 antibody, 3F8. For each patient sample a report of **(A)** Grading (proportion of cells staining positive) and **(B)** Intensity was generated by a pathologist.

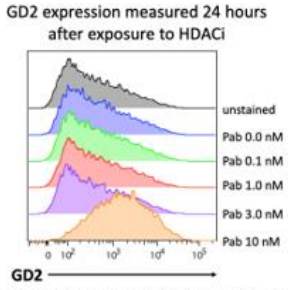


Figure 1. HDAC inhibitor [Panobinostat, 10nM] increases GD2 expression in osteosarcoma cell line (143B) after 24 hours of incubation

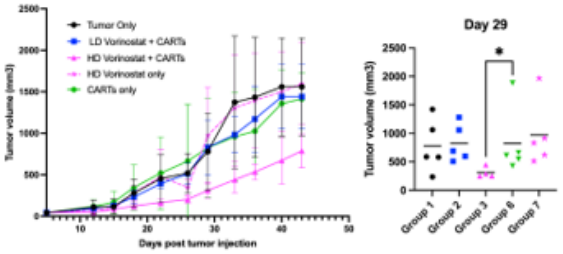


Figure 2. High dose vorinostat combined with GD2.CAR T-cells slows tumor growth compared to GD2.CAR T-cells alone; *p = 0.0338

(P 122) TARGETING CD70 USING CAR NK CELLS TO ENHANCE NK CELLS CYTOLYTIC EFFECT AGAINST OSTEOSARCOMA

Emily Rav, DO[1]; Ariana R. Anjier, MS, PhD[1]; Sunil Acharya, PhD[1]; Rafet Basar, MD[1]; Yan Zheng, PhD[1]; Shinji Maegawa, PhD[1]; Pradeep Shrestha, PhD[2]; Yifei Wang, MD[3]; Najat C. Daw, MD[2]; Katy Rezvani, MD PhD[1]; Vidya Gopalakrishnan, PhD[4]; Richard Gorlick, MD[4]; Eugenie S. Kleinerman, MD[1]; Nancy Gordon, MD[4], (1) University of Texas MD Anderson, Houston, TX, (2) MD Anderson Cancer Center, Houston, TX, (3) UT MD Anderson Cancer Center, Houston, TX, (4) The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: Osteosarcoma (OS) is the most common primary malignant bone tumor in pediatric patients primarily affecting adolescents. Cure rates for OS have not improved in over thirty years, and no new, effective therapies have been identified for metastatic disease. Natural killer (NK) cells have become an attractive tool for cancer immunotherapy. However, therapeutic efficacy has been limited in solid tumors. Thus, direct tumor targeting introducing a specific chimeric antigen receptor (CAR) may improve efficacy. Emerging knowledge suggests CD70 to be a viable target candidate in OS as expression has been correlated with tumor escape from immune surveillance and was shown to be higher in OS lung metastases. Therefore, the primary objective of this study is to assess whether using CD70 CAR-NK cells we can enhance trafficking, homing, and proliferation of NK cells and thus therapeutic efficacy against OS.

Methods: Surface expression of CD70 on human OS cell lines and normal osteoblasts was determined by flow cytometry. Western blotting was used to confirm CD70 expression by OS cell lines. Immunohistochemistry was used to analyze CD70 expression on OS patient derived xenograft tumor samples and tumor tissues from human OS mouse models. Publicly available databases were analyzed to determine CD70 mRNA expression on OS patient samples and OS cell lines compared to normal tissues. RNAseq was used to analyze CD70 mRNA expression of multiple OS cell lines. CD70 CAR constructs were sequenced, verified, and transduced into NK cells. CD70 CAR NK and control NK cells cytolytic activity against OS cells was evaluated by IncuCyte. Trafficking and cytotoxicity of CD70 CAR NK cells was analyzed in a 3D OS spheroid model. In vitro cytokine release was measured upon CD70 CAR NK/NK cells exposure to OS cells (IsoPlexis).

Results: Flow cytometry showed variable expression of CD70 on OS cells and no expression on human osteoblasts. Western blot confirmed these results on the OS cell lines tested. CD70 expression as determined by IHC, was present on tissues from OS PDX models and human OS mouse models but was found to be heterogeneous. RNAseq consistently showed increased CD70 expression in patient samples and OS cell lines compared to normal bone. There was an

increase in CD70 CAR NK percent lysis against OS cells at the highest effector:target ratios compared to control, non-transfected (NT) NK cells. CD70 CAR NK cells were able to effectively traffic to, penetrate, and exhibit cytotoxicity in a 3D OS spheroid model. We found a predominant release in the chemoattractive/stimulatory cytokines upon OS cells exposure to CD70 CAR NK cells.

Conclusion: CD70 has immunotherapy potential against OS. CD70 CAR NK cells have increased cytolytic activity against OS cells in vitro. The cytolytic activity may be influenced by the release of specific cytokines.

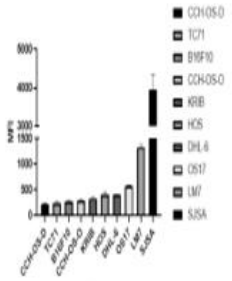


Figure 1. CD70 expression on osteosarcoma cell lines and normal human osteoblasts as determined by flow cytometry. There is variable CD70 surface protein expression (by mean fluorescence intensity [MFI]) on osteosarcoma cell lines (CCH-OS-O, CCH-OS-D and KRIB have low CD70 expression, OS17 and HOS have mid CD70 expression, and LM7 and SJSA have high CD70 expression) compared to the diffuse large B cell lymphoma cell line, DHL-6, which has known CD70 expression. TC-71 is a human Ewing sarcoma cell line and B16F10 is a murine melanoma cell line.

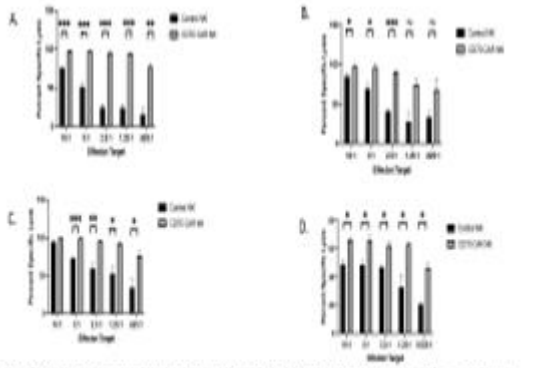


Figure 2. CD70 CAR NK cells have better cytolytic effect than control NT NK cells as demonstrated by an increase in percent specific lysis. Cytotoxicity assays were performed by co-culturing GFP-labeled osteosarcoma cells with either control NK cells or CD70 CAR NK cells at increasing effector-to-target ratios into a 96 well plate in triplicate. The GFP signal of the osteosarcoma cells was measured over time using the IncuCyte and percent specific lysis was calculated using positive and negative controls. Increased cytotoxicity is seen in OS17 (A), SJSA (B), LM7 (C), and CCH-OS-D (D). *p<0.05, **p<0.01, ***p<0.001 determined by multiple unpaired t-tests.

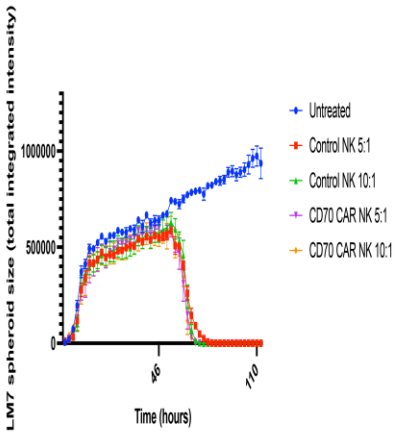


Figure 3. CD70 CAR NK cells effectively traffic to and eliminate green-fluorescence protein (GFP) labeled LM7 3D spheroids better than control NT NK cells. LM7 spheroids formed in media over 2 days were treated with a 5:1 and 10:1 effector-to-target ratio of control NT NK cells and CD70 CAR NK Cells. After 24 hours of treatment, the CD70 CAR NK cell treated spheroids had significantly decreased GFP total integrated intensity as measured by IncuCyte. Compared to untreated LM7 spheroids, CD70 CAR NK cells decrease the GFP signal in treated LM7 spheroids faster and more effectively than control NK cells.

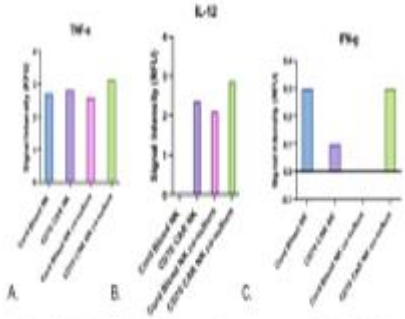


Figure 4. CD70 CAR NK cells produce more inflammatory cytokines (TNF-a, IL-12, IFN-g) compared to control cord blood NK cells when co-cultured with OS17. Using the IsoPlexis Single Cell Secretome and human NK cell cytokine panel, there was an increase in release of TNF-a (A), IL-12 (B), and IFN-g (C) by CD70 CAR NK cells when they had been co-cultured with OS17 compared to control NK cells and CD70 CAR NK cells alone.

(P 123) DEDIFFERENTIATED LIPOSARCOMAS TREATED WITH IMMUNE CHECKPOINT BLOCKADE: THE MD ANDERSON EXPERIENCE

Madeline B. Torres, MD[1]; Cheuk Hong Leung, MS[2]; Emily Z. Keung, MD, AM, FACS[3]; Chun Feng, Sr Informatics Analyst[1]; Maria A. Zarzour, MD[2]; Christopher Scally, MD[3]; Kelly Hunt, MD[3]; Anthony P. Conley, MD[2]; Andrew J. Bishop, MD[2]; B Ashleigh Guadagnolo, MD,MPH[3]; Ahsan S. Farooqi, MD PhD[4]; Devarati Mitra, MD PhD[2]; Dejka M. Araujo, MD[3]; Andy Livingston, MD, MS[3]; Ravin Ratan, MD[3]; Shreyaskumar Patel, MD[3]; Vinod Ravi, MD, MS, MBA[3]; Alexander J. Lazar, MD, PhD[3]; Christina Roland, MD, MS[3]; Neeta Somaiah, MD[3]; Elise F. Nassif, MD[1], (1) University of Texas MD Anderson Cancer Center, Houston, TX, (2) MD

Anderson Cancer Center, Houston, TX, (3) The University of Texas MD Anderson Cancer Center, Houston, TX, (4) MD Anderson Cancer Center, Sugar Land, TX

Objective: Dedifferentiated liposarcoma (DDLPS) remains a difficult tumor to treat with few treatment options, and limited response to chemotherapy and targeted therapies. Previous trials in small cohorts of patients have reported objective response rates of 14-20% in DDLPS patients treated with immune-checkpoint blockade (ICB) [1, 2].The goals of this study were to determine response rate (RR) and survival with immune checkpoint blockade (ICB) treatment, and identify clinicopathologic factors associated with response and survival to ICB-based therapy in patients with DDLPS.

Methods: We retrospectively identified all pathologically confirmed patients with DDLPS treated with ICB at The University of Texas MD Anderson Cancer Center from 01/01/2018 to 01/01/2023. Baseline patient characteristics and RECIST radiographic response to ICB treatment, as per trial definition (if on trial) were collected. Clinical variables studied included age, sex, race, ethnicity, ECOG performance status, stage, location of primitive tumor, treatment history, clinical trial participation and treatment with combination ICB. Overall survival (OS) was defined as the time from diagnosis to death or last follow-up. Progression-free survival (PFS) was defined as the time from ICB start to progression, death, or last follow-up, whichever occurred first. OS and PFS were estimated by the Kaplan-Meier method [3]. Log-rank test was used to compare the survival distribution by baseline characteristics. The hazard ratio was estimated in Cox proportional hazard regression model. All analyses were performed in SAS 9.4 and R 4.2.3.

Results: Thirty-one patients received ICB therapy for DDLPS (Table 1) with a median follow-up from start of ICB of 51.7 months. The median age at receipt of ICB was 66 years (range 30 - 82). Thirteen patients (42%) received ICB as their first line of systemic therapy, while 18 patients (58%) received ICB after at least one line of systemic therapy. Twenty-eight patients (90%) received ICB as part of a clinical trial, with the majority administered for recurrent (n = 15; 48%) and metastatic (n = 11; 35%) disease. Out of all 31 patients, best response per treating physician or clinical trial definition, was progressive (PD), stable disease (SD), and partial response (PR) in 17 (55%), 12 (39%), and 2 (6%) patients (Figure 1), respectively. Median OS was 8.5 years (95% CI 4.3, 12.2); PFS was 3.4 months (95% CI 1.6, 4.2). Median OS since best response for patients with PD and SD was 12.1 months (95% CI 1.6, not reached) and 43.5 months (95% CI 11.6, not reached) respectively. Median duration of treatment by best response for PD was 1.8 months (range 0.9 – 4.7), SD 1.2 months (range 0 – 42.7), PR 2.6 months (range 0.9 – 4.2). Median time to best response for patients with PD, SD, and PR was 1.8 months (range 1.0, 4.7), 1.3 months (range 1.1, 2.7) and range of 0.4 to 2.1 months (n = 2) respectively. There were no clinical factors associated with PFS. Patients who received systemic therapy prior to ICB had inferior OS (HR=5.15 [95% CI 1.68-15.8, p = 0.004]) than those who received ICB as first systemic therapy (Figure 2). PFS was not associated with the number of prior lines of systemic therapy.

Conclusion: Although initial reports showed promising activity of ICB in DDLPS, our experience has been disappointing in this subtype of sarcoma. Other targeted strategies in combination with immune-based therapies may enhance this response rate and should be explored. Additional translational work may identify rationale for combinations.

References

1. Tawbi HA, Burgess M, Bolejack V et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol 2017; 18: 1493-1501.

2. Chen JL, Mahoney MR, George S et al. A multicenter phase II study of nivolumab +/- ipilimumab for patients with metastatic sarcoma (Alliance A091401): Results of expansion cohorts. Journal of Clinical Oncology 2020; 38: 11511-11511.
3. Kaplan EL, Meier P. Nonparametric Estimation from Incomplete Observations. Journal of the American Statistical Association 1958; 53: 457-481.

Factor	Level	N (%)
Sex	Male	20 (65)
	Female	11 (35)
Race	Asian	1 (3)
	Black	1 (3)
	White	27 (87)
	Other	2 (6)
Ethnicity	Hispanic/Latino	5 (17)
Stage at start of ICB	Primary	5 (16)
	Recurrent	15 (48)
	Metastatic	11 (35)
Primitive tumor retroperitoneal	Yes	29 (94)
	No	2 (6)
ECOG performance status	0	13 (42)
	1	18 (58)
Number of prior lines of systemic therapy	0	13 (42)
	1 – 7	18 (58)
Number of surgical resections	0 – 1	9 (29)
	2 – 6	22 (71)
Combination ICB	Yes	11 (35)
	No	20 (65)
ICB treatment as part of clinical trial	Yes	28 (90)
	No	3 (10)

Table 1. Patient baseline characteristics. ICB: Immune check point blockade, ECOG: Easter Cooperative Oncology Group.

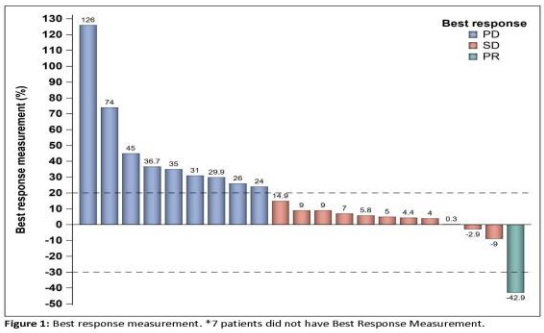


Figure 1: Best response measurement. *7 patients did not have Best Response Measurement.

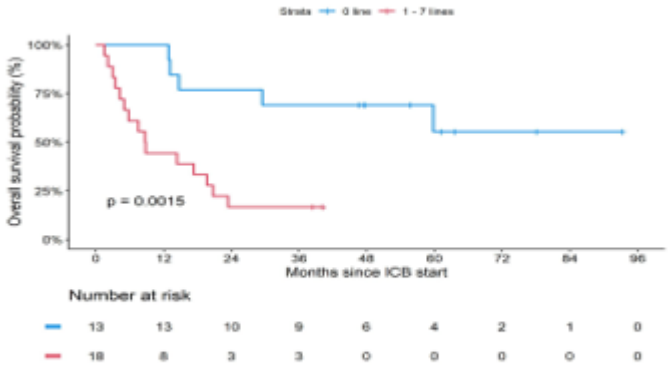


Figure 2: Overall survival curves by the number of lines of systemic therapy prior to ICB, OS is from date of ICB start for all patients.

(P 124) UNLOCKING THE POTENTIAL OF MEASLES VIRUS ONCOLYTIC IMMUNOTHERAPY FOR SARCOMA: BIOMARKER DISCOVERY AND THERAPEUTIC VACCINE DEVELOPMENT

Priya Chudasama, PhD[1]; Nicolas Duus[2]; Rajesh Pal, Ph.D.[1]; Maximiliane Finkbeiner, M.Sc.[3]; Nicola Biondi, M.Sc.[1]; Patrick Schmidt, Ph.D.[4]; Guy Ungerechts, M.D.[5]; Stefan Fröhling, MD[6]; Christine Engeland, M.D.[7], (1) Precision Sarcoma Research Group, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Heidelberg, Baden-Wurttemberg, Germany, (2) Precision Sarcoma Research Group, German Cancer Research Center (DKFZ) and Clinical Cooperation Unit Virotherapy, National Center for Tumor Diseases (NCT) Heidelberg, Heidelberg, Baden-Wurttemberg, Germany, (3) Clinical Cooperation Unit Virotherapy, German Cancer Research Center (DKFZ) and Experimental Virology, Center for Biomedical Research and Education, Witten/Herdecke University, Heidelberg, Baden-Wurttemberg, Germany, (4) Clinical Cooperation Unit Applied Tumor Immunity, German Cancer Research Center (DKFZ), Heidelberg, Baden-Wurttemberg, Germany, (5) Clinical Cooperation Unit Virotherapy, German Cancer Research Center (DKFZ) and Department of Medical Oncology, Heidelberg University Hospital, Heidelberg, Baden-Wurttemberg, Germany, (6) Department of Translational Medical Oncology, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center (DKFZ), Heidelberg, Baden-Wurttemberg, Germany, (7) Clinical Cooperation Unit Virotherapy, German Cancer Research Center (DKFZ) andExperimental Virology, Center for Biomedical Research and Education, Witten/Herdecke University, Heidelberg, Baden-Wurttemberg, Germany

Objective: The objective of this preclinical study is to advance immunotherapy for sarcoma using oncolytic measles virus (MeV). MeV are promising therapeutic vectors due to their preferential replication in malignant cells, immunogenic tumor cell death leading to priming of anti-tumor immunity, a versatile reverse genetics system and an excellent safety record. In this study, we aim to identify predictive biomarkers for the sensitivity of sarcoma to measles virotherapy, assess potential sub-entity specific differences and demonstrate a proof-of-concept for using tumor-associated antigen (TAA)-encoding MeV as a therapeutic vaccine for the treatment of sarcoma.

Methods: A panel of 17 sarcoma cell lines representing 5 sarcoma sub-entities (leiomyosarcoma, liposarcoma, synovial sarcoma, osteosarcoma, and undifferentiated pleomorphic sarcoma) were used to assess the receptor expression, viral gene expression, production of viral progeny and virus-mediated cytotoxicity across different sarcoma subtypes. Based on the outcome, cell lines were stratified into high sensitive and low sensitive groups. This was followed by transcriptomic analyses to uncover underlying pathways and predictive gene signatures contributing to differences in sensitivity towards measles virotherapy. Additionally, we designed and produced recombinant MeV encoding two variants of the NY-ESO-1 TAA: MeVac ld-NY-ESO-1 containing the complete coding sequence of NY-ESO-1, and MeVac ld-Ub-SLLMWITQCx6 featuring an epitope cassette harboring the known immunodominant HLA-A*02:01 restricted epitope NY-ESO-1-157–165. To evaluate whether this construct may act as an oncolytic vaccine to enhance antigen-specific T cell-mediated killing, NY-ESO-1-TCR+ T cells were generated from HLA-A*02:01-positive donors and co-cultured with MeVac ld-NY-ESO-1 or MeVac ld-Ub-SLLMWITQCx6 infected sarcoma cells. Tumor cell killing was monitored using xCELLigence technology.

Results: Flow cytometry showed high expression of the main MeV entry receptor CD46 on all 17 investigated cell lines. Cytotoxicity post-MeV infection was also observed in all sarcoma cell lines, underlining the applicability of oncolytic MeV approaches for a broad spectrum of sarcoma sub-entities. Interestingly, varying levels of viral protein and progeny production as well as virus-mediated cytotoxicity after MeV infection were observed across cell lines which were not sub-entity dependent, suggesting a requirement for a systematic investigation of the cellular response to MeV. Comparative transcriptomic analysis revealed enrichment of cell cycle, mitosis, angiogenesis, and DNA repair pathways in highly sensitive sarcoma cell lines, and inflammatory, interferon and cytokine response pathways in those with low sensitivity. Additionally, we successfully cloned and produced the two recombinant MeVs, i.e, MeVac ld-NY-ESO-1 and MeVac ld-Ub-SLLMWITQCx6. These viruses were shown to markedly enhance antigen-specific T cell-mediated killing of sarcoma cells when co-cultured with NY-ESO-1-TCR+ T cells.

Conclusion: Our ongoing study highlights the potential of measles virotherapy to both mediate direct tumor cell lysis, as well as enhance anti-tumor immune response as oncolytic vaccine in multiple sarcoma sub-entities. Currently, we are refining MeV sensitivity signature by integrative transcriptomics and proteomics analysis that may act as a predictive biomarker for the response to measles virotherapy. In an in vitro setting, we demonstrate that an oncolytic MeV vaccine carrying NY-ESO-1 can enhance the T cell-mediated killing of sarcoma cells, thereby providing a successful proof-of-concept for the application of MeV-based oncolytic vaccine.

(P 125) MYELOMODULATORY TREATMENTS AUGMENT THE THERAPEUTIC BENEFIT OF ONCOLYTIC VIRUS IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS BY MODULATING THE TUMOR MICROENVIRONMENT

Siddhi Nath Paudel, PhD[1]; Brian Hutzen, PhD[2]; Chun-Yu Chen, PhD[2]; Katherine Miller, PhD[2]; Mark A. Currier, MS[2]; Pin-Yi Wang, PhD[2]; Andrea M. Glaspell, BS[2]; Timothy P. Cripe, MD, PhD[2], (1) Nationwide Childrens Hospital, Columbus, OH, (2) Nationwide Children's Hospital, Columbus, OH

Objective: The immunosuppressive microenvironment remains a key challenge for advancing immune-based strategies in targeting solid tumors. This study aimed to investigate whether

rational targeting of the myeloid cells in Malignant Peripheral Nerve Sheath Tumors (MPNST) could potentiate the effectiveness of oncolytic herpes simplex viroimmunotherapy.

Methods: We employed whole genome RNA sequencing to characterize murine MPNST models at baseline. In two preclinical mouse models of MPNST, we combined the oncolytic virus T-VEC with myeloid-cell targeting therapeutics, Pexidartinib or Trabectedin, to assess the combination therapy's antitumor efficacy and survival benefits. Antibody-induced cell depletion was utilized to evaluate if the observed survival benefits were immune-mediated. Additionally, Flow cytometry was employed to investigate treatment-induced changes in the tumor microenvironment, including immune cell infiltration and immune cell phenotypes.

Results: Monotherapy with oncolytic herpes simplex viruses demonstrated modest efficacy. However, combining T-VEC with myelomodulatory therapies, namely pexidartinib or trabectedin, significantly increased survival in immunocompetent C57BL/6 or Balb/c tumor models, respectively, albeit complete tumor regressions were not achieved. Notably, the survival benefit diminished upon combining T-VEC and Trabectedin with antibodies targeting CD4 and CD8 T cells, underscoring the dependence on CD4 and CD8 T cells for the enhanced therapeutic effect. Subsequent flow cytometry analysis of mice treated with the T-VEC and trabectedin combination revealed a notable increase in the proportion of tumor-associated retrovirus antigen-specific CD8 T cells, targeting the envelope glycoprotein gp70, compared to monotherapy groups receiving T-VEC or trabectedin alone. Furthermore, there was a decrease in the proportions of M2 macrophages and myeloid-derived suppressor cells while increasing the M1/M2 ratio within the virus-treated and combination therapy cohorts.

Conclusion: Our findings provide compelling evidence that myelomodulatory therapies can augment the antitumor T-cell response and improve the therapeutic benefits of oncolytic virotherapy in murine models of MPNST. These results provide valuable insights for designing and implementing future immunotherapeutic strategies in the management of MPNST.

Improving Sarcoma Care Globally: Access, Equity, Advocacy and Policy

(P 126) CURRENT PRACTICE IN SPERMATIC CORD SARCOMA: ONCOLOGICAL TIMELINES AND OUTCOMES ACCORDING TO INITIAL RESECTIONS PERFORMED AT A LOCAL HOSPITAL VERSUS EARLY PATIENT REFERRAL TO A TERTIARY CENTRE.

Misbah Khan, MD, FCPS, FEBS-SO[1]; Marco Baia, MD[2]; Marco Fiore, MD, FEBS, FACS[3]; Chiara Colombo, MD[4]; Dario Callegaro, MD[2]; Sandro Pasquali, MD, PhD[2]; Alessandro Gronchi, MD, FSSO[5]; Stefano Radaelli, MD[6], (1) ESHT NHS foundation trust, Saint Leonards on sea, England, United Kingdom, (2) Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (3) Department of Surgery, Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (4) Department of Surgery, Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (5) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (6) Sarcoma Service, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy

Objective: Spermatic cord sarcoma remains an exceedingly rare yet highly heterogeneous disease with an annual incidence of 0.3 cases per million men. Hence, an appropriate risk assessment and personalized treatment necessitate multidisciplinary care in referral centres for soft tissue sarcoma. Nevertheless, most patients receive inadequate surgery in local hospitals following the presentation of an asymptomatic inguino-scrotal lump and diagnosis is mainly determined postoperatively.

This retrospective study looked at the oncological outcomes of inguino-scrotal sarcoma cases managed with curative intent resection at a tertiary referral sarcoma centre. We specifically studied how inadequate resections impact the oncologic outcomes compared to primary tumour resections at the reference centre.

Methods: One hundred twenty-five consecutive patients affected by primary, localized spermatic cord sarcoma surgically managed at our reference centre from January 1982 through January 2021 were included.

Primary end-point measures the rate of disease relapse, with disease-free (DFS) and overall survival (OS) rates defined. These outcomes were evaluated with multi-nomial logistic regression and Cox proportional hazards regression models for a co-relation to the defined variables of age, tumour size, pathologic grade, resection margin status, initial inadequate surgery, adjuvant treatment given and the time interval from onset of symptoms to oncologic resection. We also compared the treatment intervals among the subgroups of primary versus re-resection surgery, along with defined patient, tumour and treatment-related variables (Table-1).

Results: Eighty-eight patients (70.4%) had their initial diagnosis established on an inadequate surgical excision performed in a local hospital, followed by a re-excision at our centre (Re-resection group). At the same time, 37 (29.6%) were managed primarily with biopsy and treatment at our reference centre (Primary-resection group).

The two groups showed statistically significant differences in tumour size distribution, histopathology, and postoperative complication rate ($p< 0.05$). However, a prompt referral after an initial surgery on a non-cancer pathway did not result in a statistically significant effect on treatment intervals (Table-1, Figure-1). In patients undergoing re-excision after inadequate primary resection, residual disease was found in 44 (50%) specimens. Following R0 resection, there was no statistically significant difference in the risk of recurrence between the two groups ($p=0.34$) (Table-2).

The rate of local recurrence (LR) and distant metastasis (DM) for the whole cohort was 11.2% each over a median follow-up of 54 months (IQR 25 -105). The cohort's local relapse-free and distant metastasis-free survival were 88.7% and 87.4%, respectively. Higher tumour grade and a marginal (R1) resection were associated with worse DMFS and LRFS, respectively ($p=< 0.05$) (Figure- 2).

5-year DFS was 78.9% for the study population, 51.6% in G3, 76.2% in G2 and 97.5% in G1 FNCLCC ($p=0.001$).

5-year OS was 91.8 % in the whole cohort and 66.1% in G3 FNCLCC, compared to 94.8% and >99.5% in G2 and G1 FNCLCC tumours, respectively ($p=0.054$).

Conclusion: Prompt referral to a tertiary centre, where multidisciplinary evaluation and an oncologically sound surgical resection is the standard of treatment, has the ability to align the OS and DFS of patients receiving incomplete surgery elsewhere to those treated primarily in referral centres. Nevertheless, the primary determinant of prognosis remains surgical margin and tumour grade for LRFS and DMFS, respectively.

Table 1: Demographic variables in each group

	Total population	Primary resection group	Re-resection group	Uni-variate
	Number(Percentage) n(%)	Number(Percentage) n(%)	Number(Percentage) n(%)	P- value
		37(29.6%)	88(70.4%)	
	Mean +- SE	Mean +- SE	Mean +- SE	
Age in years	61.3 +-1.31	63.3 +-1.4.1	60.5 +-1.4.7	0.315
Symptoms at Presentation				
Mass	119(95.2%)	34(91.9%)	85(96.6%)	
Incidental finding	2(1.6%)	1(2.7%)	1(1.1%)	0.739
Pain	2(1.6%)	1(2.7%)	1(1.1%)	
Tumour size at initial surgery in cm	8.14 +- 0.5	10.5 +- 0.9	7.2 +- 0.5	0.024
Tumour side				
Right	66(52.8%)	20(54.1%)	46(52.3%)	0.855
Left	59(47.2%)	17(45.9%)	42(47.7%)	
Histopathology*				
WDLPS	42(32.6%)	9(24.3%)	33(37.5%)	
DDLPS	57(45.6%)	24(64.9%)	33(37.5%)	0.043
LMS	14(11.2%)	2(5.4%)	12(13.6%)	
Other Sarcoma	12(9.6%)	2(5.4%)	10(11.4%)	
FNCLCC				
Grade1	43(34.4%)	9(24.3%)	34(38.6%)	
Grade2	53(42.4%)	19(51.4%)	34(38.6%)	0.276
Grade3	29(23.2%)	9(24.3%)	20(22.7%)	
Adjuvant Treatment				
No	103(82.4%)	29(78.4%)	74(84.1%)	0.444
Yes	22(17.6%)	8(21.6%)	14(15.9%)	
Adjuvant Treatment (Yes)**				
Pre-Op CT	5(4.0%)	1(2.7%)	4(4.5%)	
Post-Op CT	5(4.0%)	2(5.4%)	3(3.4%)	
Pre and Post-OP CT	1(0.8%)	1(2.7%)	0(0.0%)	
Pre-Op RT	4(3.2%)	2(5.4%)	2(2.3%)	
Post-Op RT	5(4.0%)	1(2.7%)	4(4.5%)	
Pre-Op CRT	1(0.8%)	1(2.7%)	0(0.0%)	
Post-Op CRT	1(0.8%)	0(0.0%)	1(1.1%)	
Peri-Operative outcomes				
Complications (Clavien-Dindo Grade)	17(13.6%)	11(29.7%)	6(6.8%)	
2	11(8.8%)	6(16.2%)	5(5.7%)	0.004
3b	5(4.0%)	4(10.8%)	1(1.1%)	
4b	1(0.8%)	1(2.7%)	0(0.0%)	
Mesh repair	84(67.2%)	29(78.4%)	55(62.5%)	0.084
Vascular reconstruction	3(2.4%)	3(8.1%)	0(0.0%)	0.007
Plastic reconstruction	7(5.6%)	3(8.1%)	4(4.5%)	0.429
Duration of Surgery in minutes	138.6 +- 6.7	177.9 +- 15.0	121.7 +- 6.4	<0.001
Treatment intervals.				
Length of Follow up in months	67.7 +-5.3	49.3 +- 6.2	75.4 +- 6.9	0.103
Time from symptoms to diagnosis in weeks	56.6 +-10.0	49.9 +-16.5	59.4 +-12.5	0.631
Time from diagnosis to Surgery in weeks	19.5 +-0.8	17.0 +- 1.6	20.5 +- 0.9	0.258
Time from symptoms to Oncologic resection with curative intent in weeks	76.1 +-10.1	66.9 +-16.3	79.9 +- 12.5	0.588

*WDLPS= Well differentiated liposarcoma, DDLPS= Dedifferentiated liposarcoma, LMS= Leiomyosarcoma, Others= rhabdomyosarcoma(4), undifferentiated pleomorphic sarcoma(4), synovial sarcoma(2), and one each of solitary fibrous tumour and myxofibrosarcoma.
** CT= Chemotherapy, RT= Radiotherapy, CRT= Chemoradiotherapy.

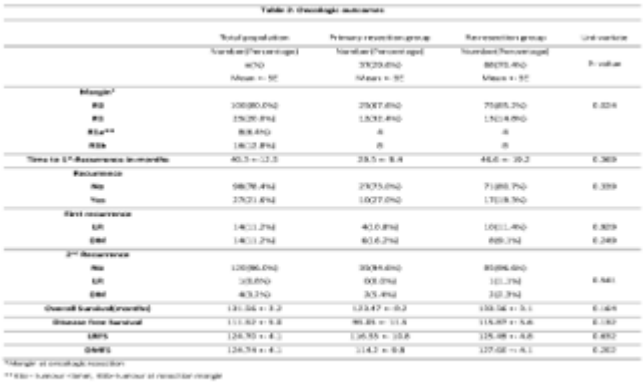


Figure 1. Distribution of treatment intervals between two groups.

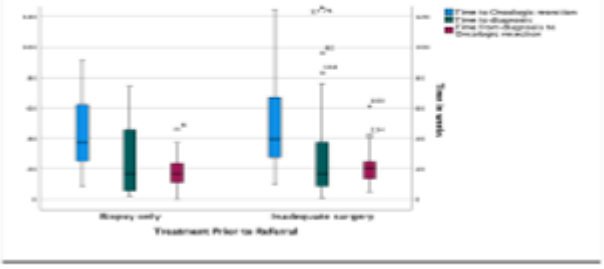
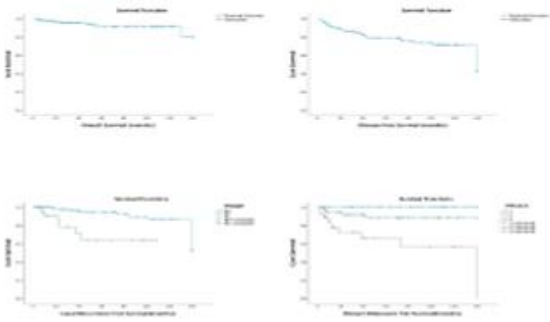


Figure 2: a) Overall Survival b) Disease free Survival c) Local relapse-free and d) distant metastasis-free survival.



(P 127) **TRENDS IN CONTACTS TO THE SARCOMA UK SUPPORT LINE**

Helen F. Stradling, BNurs (Hons), MSc, Sarcoma UK, London, England, United Kingdom

Objective: The Sarcoma UK Support Line (SL) was opened in February 2016 with the aim of offering free, confidential advice and support for anyone affected by sarcoma. The team consists of sarcoma nurses and advisors with experience of sarcoma which ensures that people accessing the team receive up to date and clinically relevant information. Since opening the team have seen a consistent increase in the number of contacts, a contact being an

interaction via telephone, email, text or social media, but have the trends and reasons for contact remained the same over the last seven years?

Methods: The SL team record all contacts on a secure database and an annual audit is undertaken in which numbers are compared year on year. This allows the team to notice trends and changes as well as show the real impact of the SL. In 2020 the SL team started a 'direct referrals' service with the Swansea clinical team, whereby the Swansea team email the SL once a diagnosis has been made and with consent they share the persons contact details. The SL team then make contact with the individual and offer their support. This process not only allows the SL team quicker and more direct access to people affected by sarcoma, but also removes some of the 'support' pressure on the clinical teams. This service has now been expanded and is available to 7 clinical teams. In January 2023 the SL expanded and started to have two members of the team on the line each day, allowing more calls to be answered at the first time of trying and less people needing to leave voicemails for the team.

Results: Since the opening of the SL the team have had over 19,000 contacts with more than 4,000 individuals. The 2022/23 year saw an increase in contacts - 4622, compared to the 2016/17 year of opening of 947, an increase of over 380%. The number of contacts relating to palliative treatments increased significantly in the summer of 2020, with the country going into lockdown, and the need to cease palliative chemotherapy for all due to the pandemic was difficult for all concerned. That same year the team had increased contacts regarding the changes to follow up, postponed scans, how the lockdown would affect people and their care as well as an increase in contact from worried family and friends. The team have seen a trend of the top 3 reasons for people making contact as being support, treatment and diagnosis up until the 2022/23 year in which the contacts in the the pre-diagnosis stage overtook those at the diagnosis stage and became the 3rd most popular reason for people making contact. Email continues to be the method of contact of choice, however, the 2022/23 year has seen an increase in the amount of time the team have spend on the telephone, with an increase on the previous year of 64% with 450.65 hours and an average call time of 23 minutes. This year has also seen an increase in the number of contacts at the referral stage, with the team needing to move away from the directing people to advocate for themselves, into a more direct contacting of the clinical teams on peoples behalf to ensure referrals were being made and being received by the appropriate teams. The team have spent an increasing amount time over the last 3 years to give people the words to use when trying to access health care services to ensure any worrying symptoms are not ignored, this was not so much of an issue prior to 2020 and the COVID pandemic.

Conclusion: The Sarcoma UK SL is available for anyone affected by sarcoma and strive to be in contact with as many people as possible at the earliest opportunity. The team are a beacon in hearing how things really are for people and are able to escalate issues not only within Sarcoma UK, but nationally with NHSE, and other charity collaborations. The team aim to increase the direct referral scheme and hope to open this to 10 clinical teams by the end of the 2023/24 year to allow us to increase our reach. The teams links with the clinical sarcoma community cannot be underestimated and in a time of intense pressure within the NHS the SL team are able to offer an extra support to people affected by sarcoma and time, which is hard for our clinical colleagues to find.

(P 128) **THE IMPACT OF HISPANIC ETHNICITY IN PRESENTATION AND OUTCOMES OF PATIENTS WITH SYNOVIAL SARCOMA**

Priscila Barreto coelho, MD[1]; Caroline Hana, MD[2]; Natalia Yanchenko, MD[3]; Steven Bialick, DO[4]; Andrew Rosenberg, MD[3]; Aditi Dhir, MD[5]; Emily Jonczak, MD[6]; Gina Z. D'Amato, MD[7]; Atif Hussein, MD[8]; Jonathan Trent, MD, PhD[9], (1) University of Miami, Miami, FL, (2) Memorial Healthcare Hospital, Miami, FL, (3) Sylvester Comprehensive Cancer Center, MIAMI, FL, (4) Sylvester Comprehensive Cancer Center, University of Miami, Deerfield Beach, FL, (5) University of Miami Miller School of Medicine, Miami, FL, (6) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL, (7) Sylvester Comprehensive Cancer Center, Miami, FL, (8) Memorial Healthcare Hospital, Hollywood, FL, (9) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL

Objective: Synovial sarcomas (SS) harbor a specific, balanced, reciprocal translocation t(X;18) leading to the oncogenic SS18-SSX fusion. It accounts for 5-10% of all soft tissue sarcomas, and there is a lack of information regarding the impact of race and ethnicity on its presentation and outcomes. Florida is currently not included in the SEER database, and it is the US's ninth-most diverse population, with a diversity index of 64.1% and with the sixth-highest Hispanic population among all states. Sylvester Comprehensive Cancer Center (SCCC) and Memorial Healthcare System (MHS) are two large oncology centers in South Florida that provide care for this rare type of tumor. We aimed to identify the impact of Hispanic ethnicity in SS.

Methods: We retrospectively reviewed all patients with a confirmed diagnosis of SS between January 2003 and January 2022 in SCCC and MHS. All data were retrieved from electronic medical records. SPSS Statistics was utilized, Fisher exact test and log-rank test were used for analysis. A two-sided $p< 0.05$ was considered statistically significant.

Results: A total of 73 patients had a pathologically confirmed diagnosis of SS, with information regarding ethnicity, during the time period evaluated, 45.3% were Hispanic, and 54.7% were non-Hispanic. The Hispanic group was comprised mainly of female patients (66%) with a median age of diagnosis of 44 years old, these findings were similar in the non-Hispanic group, where 55% were women, and the median age at diagnosis was 42 years old. Fewer patients identified themselves as Black within the Hispanic group (3%) compared to the non-Hispanic (22%) (p=0.016). The most common site of primary disease was the extremity in both groups (Hispanic 63% vs non-Hispanic 75%) (p=0.384). Most tumors were diagnosed with a size bigger than 5cm (Hispanic 57% vs non-Hispanic 65%) (p=0.577) and with pathology reporting a monophasic SS (Hispanic 45% vs non-Hispanic 50%) vs biphasic or poorly differentiated (p=0.061). Non-Hispanics had more poorly differentiated tumors (Hispanic 3% vs non-Hispanic 17.5%) (p=0.054). Hispanic patients did have fewer cases of metastatic disease at diagnosis (Hispanic 9% vs non-Hispanic 32%) (p=0.023). The median overall survival (mOS) among Hispanic patients was not reached in our study, and mOS among the non-Hispanic patients was 5 years (p=0.099).

Conclusion: In our study, the patients more commonly presented with large tumors (>5cm) in the extremity and monophasic histology, regardless of their ethnicity. Non-Hispanic patients more often had poorly differentiated tumors, metastatic disease at diagnosis, and a trend toward worse mOS. This study had limited power due to the limited number of patients, and further studies are needed to identify the presence of disease-related differences in these populations.

(P 129) LOW RATES OF SERVICE CONNECTIVITY FOR SOFT TISSUE SARCOMA (STS) AMONG ELIGIBLE VIETNAM VETERANS WITH AGENT ORANGE EXPOSURE

Sarah E. Lindsay, MD[1]; Cecelia Madison, MS[2]; Reid Thompson, MD, PhD[1]; Duncan Ramsey, MD, MS[1]; Yee-Cheen Doung, MD[1]; Kenneth R. Gundle, MD[2], (1) Oregon Health & Science University, Portland, OR, (2) Portland VA Medical Center, Portland, OR

Objective: The Veterans Heath Administration (VHA) is the largest integrated health care system in the United States, caring for over 9 million Veterans. The Veterans Administration (VA) offers a disability program to compensate veterans for conditions incurred due to their service (service-connected conditions). This compensation can include a monthly payment as well as cost-free medical care for the condition. Veterans who served in Vietnam or within twelve nautical miles of Vietnam shores between January 1962 and May 1975 are presumed to have Agent Orange exposure. The VA presumes by law that a number of diagnoses including respiratory cancers and prostate cancer are related to Agent Orange or other herbicide exposure during military service. Soft tissue sarcoma (STS) is also on the VA presumptive list for Agent Orange and herbicide exposure (1), but the rarity of STS may be a barrier for patients and their care teams to recognize and begin a VA disability claim. With the recent passage of the Promise to Address Comprehensive Toxics (PACT) Act, the VA has expanded service-connected health benefits for Veterans exposed to burn pits and other toxic substances. It is currently unknown whether Veterans with STS are receiving the service-connected benefits for which they are eligible. The purpose of this study was to evaluate whether Veterans with a diagnosis of STS have been service connected for their cancer. We hypothesized the Vietnam Veterans with a recognized exposure to Agent Orange would have a high rates of service connection for STS.

Methods: Institutional Review Board approval was obtained for this retrospective study. Through the National VA Corporate Data Warehouse, all surgical pathology notes were identified from 2003 to 2022. These reports were retrospectively searched for the terms “myxofibrosarcoma”, “dedifferentiated liposarcoma”, “myxoid liposarcoma”, and “undifferentiated pleomorphic sarcoma (UPS)”. These diagnoses represent the most common STS (2). Manual review of the pathology reports facilitated creation of a cohort of Veterans with confirmed STS. Era of service, service-connected conditions, and information on Agent Orange exposure were abstracted from the data warehouse. All service-connected disability conditions for patients in the cohort were reviewed to determine whether or not these conditions may be related to STS. Examples of these conditions include malignant neoplasm of the respiratory system, or malignant growth of the bones. Proportions of groups were compared by Chi-square or Kruskal-Wallis tests.

Results: Over 10.5 million pathology reports were searched using the diagnosis terms. Manual review of pathology notes confirmed 200 myxofibrosarcoma, 148 dedifferentiated liposarcoma, 119 myxoid liposarcoma, and 257 undifferentiated pleomorphic sarcoma (UPS) cases in our cohort. Of the 724 total Veterans in our cohort, 62 (8.5%) were specifically service-connected for STS. There was no difference in STS service condition by sarcoma subtype ($p=0.53$). Of the 6,786 non-sarcoma service-connected disability conditions for patients in our cohort, 232 (5%) were determined to be likely related to STS. One hundred and thirty-two (18%) patients were identified in the medical record as having exposure to Agent Orange. One hundred and twenty-eight of these patients were exposed to Agent Orange in Vietnam, three were exposed in the Blue Water Navy (BWN), and one was exposed in the Korean Demilitarized Zone (DMZ). Veterans with recognized Agent Orange exposure were more likely to be service-connected for their STS (19% vs 2.5%, $p< 0.0001$). Service-connectivity varied significantly by era of service, and era with the highest proportion of STS service-connectivity was Vietnam (12%, $p=0.0006$). Vietnam Veterans with recognized Agent Orange exposure were more likely to be service-

connected for their STS (29.5% vs 4%, p< 0.0001). However, 92/132 (70%) of Vietnam Veterans with recognized Agent Orange exposure and a STS diagnosis were not specifically service-connected for sarcoma.

Conclusion: Although STS is a service-connected diagnosis for Agent Orange exposure, in the present study we found low rates of service connectivity for eligible Veterans. These data suggest that the majority of eligible patients are not receiving disability compensation to which they are entitled. Following the passage of the PACT Act, there are additional Veterans who are eligible for disability compensation for a range of conditions. Monitoring the rates of actual receipt of service connection and its documentation is one means of assessing the efficacy of these programs. Physicians caring for Veterans with STS may be able to assist Veterans by encouraging them to file disability claims. Level of Evidence: Diagnostic Study, Level II Works Cited: 1. U.S. Department of Veterans Affairs. Soft Tissue Sarcoma and Agent Orange. Available at: https://www.publichealth.va.gov/exposures/agentorange/conditions/soft-tissue-sarcoma.asp, accessed March 14, 2023. 2. Stacchiotti, S. et al. Ultra-rare sarcomas: A consensus paper from the Connective Tissue Oncology Society community of experts on the incidence threshold and the list of entities. Cancer 127, 2934–2942 (2021).

(P 130) THE ADOPTION OF MEDICAID EXPANSION IN THE AFFORDABLE CARE ACT IS NOT ASSOCIATED WITH STAGE AT PRESENTATION OR 5-YEAR OVERALL SURVIVAL FOR BONE OR SOFT TISSUE SARCOMAS

Sarah E. Lindsay, MD[1]; Yee-Cheen Doung, MD[1]; Kenneth R. Gundle, MD[2]; James Hayden, MD, PhD[1]; Duncan Ramsey, MD, MS[1], (1) Oregon Health & Science University, Portland, OR, (2) Portland VA Medical Center, Portland, OR

Objective: Despite significant advances in healthcare in the United States, inequity persists. The primary purpose of the 2010 Patient Protection and Affordable Care Act (ACA) was to address this inequity by increasing access to healthcare for uninsured individuals by subsidizing private insurance and, in participating states, by expanding Medicaid insurance. Patients with private and Medicaid coverage are more likely than those without insurance to receive preventative care and cancer screening. Uninsured patients are more likely to be diagnosed with late-stage cancer. Implementation of the ACA has been associated with higher rates of early-stage diagnosis and improved mortality for a number of cancers types. Early-stage diagnosis of bone and soft tissue sarcomas improved significantly in states that adopted Medicaid expansion compared to states that did not. However, Medicaid patients with bone and soft tissue sarcomas have been shown to have reduced disease-specific survival, higher rates of distant metastases, and higher rates of amputation compared to those with non-Medicaid insurance. To date, the implications of Medicaid expansion on long-term sarcoma survival have not been assessed. The purpose of this study was to assess the association of Medicaid

expansion on stage at presentation and 5-year overall survival (OS) for bone and soft tissue sarcomas.

Methods: The National Cancer Database (NCDB) is the largest cancer registry in the world and offers validated, standardized collection methods that are crucial for the accurate investigation of cancer outcomes. We queried the NCDB for all patients diagnosed with bone and soft tissue sarcomas from 2011-2013 and 2015-2022 residing in states that either expanded Medicaid on January 1, 2014 or did not expand Medicaid. Year of diagnosis, state of diagnosis, age, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other/unknown), and Charlston/ Deyo comorbidity index were abstracted. Additionally, zip code, median household income, and high school attainment were used to categorize patients into quartiles based on US Census data. Outcomes of interested included stage at presentation and 5-year OS. A difference-in-difference analysis was used for evaluating stage, and a multivariable Cox proportional hazards analysis was used to assess OS.

Results: For soft tissue sarcoma, living in a state that opted into expansion was associated with a small improvement in 5-year OS (p=0.008) all times points; however, the implementation of the expansion itself in 2014 was not temporally associated with stage at presentation or improvements in 5-year OS as a discrete (pre- and post-2014) or continuous variable. For bone sarcoma, none of the studied variables were associated with stage at presentation or 5-year OS.

Conclusion: For bone and soft tissue sarcomas, the adoption of Medicaid expansion as part of the ACA is not associated with changes in stage at presentation or 5-year OS. Investigation into the reasons for better 5-yr OS both pre- and post-expansion for patients residing in states that chose to adopt Medicaid expansion is warranted to improve survival for sarcoma patients.

(P 131) PATIENT-REPORTED CONTINUED BENEFITS IN PATIENTS TREATED WITH PEXIDARTINIB FOR TENOSYNOVIAL GIANT CELL TUMOR BASED ON A REAL-WORLD STUDY IN THE UNITED STATES

Dong Dai, PhD, MBA[1]; Feng Lin, Ph.D.[1]; Irene Pan, MSc[2]; Klaus Freivogel, n/a[3]; Xin Ye, PhD[1]; Kristen Tecson, PhD[1]; William D. Tap, MD[4], (1) Daiichi Sankyo, Inc., Basking Ridge, NJ, (2) United BioSource LLC, Blue Bell, PA, (3) United BioSource LLC, London, England, United Kingdom, (4) Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Tenosynovial giant cell tumors (TGCT) are a family of neoplastic, inflammatory lesions that arise within the synovium and can be locally aggressive. Patients with TGCT frequently experience pain and joint swelling, as well as stiffness, reduced range of motion, and instability in affected joints. Disease progression can lead to additional complications, most commonly mobility impairment and joint deterioration. Pexidartinib (TURALIO), a CSF-1R antagonist, is the first and only systemic therapy approved by the FDA for the treatment of adult patients with symptomatic TGCT associated with severe morbidity or functional limitations and not amenable to improvement with surgery. The objective of this study was to assess symptom change over time among patients who were treated with pexidartinib for TGCT in a real-world setting.

Methods: This was a longitudinal observational study of patients receiving pexidartinib for TGCT treatment in the real-world setting. The study collected Patient Reported Outcomes (PRO) from patient surveys prospectively, and clinical data from medical charts retrospectively. The surveys were administered to adult patients enrolled in the post-market Risk Evaluation

and Mitigation Strategy (REMS) program: the first wave was sent in 2021 (“Baseline”), and the final was sent in 2022 (“Follow Up”). Patients were required to be receiving pexidartinib before the baseline survey and to be on pexidartinib when they accessed the follow up survey. The surveys consisted of validated instruments that measured the following domains: (1) physical function of the upper/lower extremity by Patient-Reported Outcomes Measurement Information System physical function (PROMIS-PF); (2) joint stiffness by the one-item Worst Stiffness Numeric Rating Scale (NRS); (3) joint pain by the one-item Brief Pain Inventory (BPI) Worst Pain Numeric Rating Scale Score (NRS); (4) treatment satisfaction by the 14-item Treatment Satisfaction Questionnaire for Medication (TSQM); and (5) change in physical function and overall symptom since initiating pexidartinib by the Patient Global Impression of Change (PGIC). The EQ-5D-5L, which measures five dimensions of health state, was additionally administered in the follow-up survey to understand patients’ general health state. The scores and paired changes from baseline were summarized by descriptive statistics and a random slope regression model was developed to adjust for the time since first pexidartinib dose.

Results: At baseline (2021), 83 participants completed the first survey. A second survey was sent to the subset of 45 patients who remained on pexidartinib treatment in 2022; 31 patients completed the follow up survey. The mean (SD) time between baseline and follow up was 1.02 (0.17) years, mean (SD) age at the follow up was 41.9 (13.70) years, and 67.7% were female. The most common tumor sites were in the knee (67.7%) or ankle (16.1%). Majority of patients (59.0%) at baseline reported ‘much improved’ or ‘very much improved’ physical function with pexidartinib treatment compared to life before pexidartinib treatment (Table 1). Further, there was a non-significant change in PROMIS-PF score from baseline to follow up with mean (SD) of -0.43 (5.724) (Table 2), indicating stability of physical function improvement over time while on therapy. At the baseline survey, improvements were reported during pexidartinib treatment with a mean (SD) change of -3.2 (3.1) in worst stiffness NRS and -3.0 (2.7) in worst pain NRS when compared with that prior to pexidartinib treatment (Table 1). Results were sustained between the baseline and follow up, with a mean (SD) change of worst stiffness NRS of 0.8 (2.67) and a mean (SD) change of worst pain NRS of -0.1 (2.09); neither was significant (Table 2). The only TSQM metric that changed significantly over time was the TSQM Effectiveness Domain, of which the mean (SD) was 68.64 (19.167) at baseline and 73.75 (17.749) in the follow up, an average paired increase of 7.28 (19.021) (p-value=0.04) (Table 2). In the follow up, at least 71% of respondents indicated that they had only slight or no problems at each of the five domains of the EQ-5D-5L (Table 3). And majority (85.7%) of patients reported improved overall symptoms since the start of the study (Table 4).

Conclusion: In this study, most TGCT patients reported symptom improvement in physical function, stiffness and pain during pexidartinib treatment than prior to pexidartinib treatment at baseline. After an additional year of follow up, the PROs of physical function, worst stiffness, worst pain, and treatment satisfaction were sustained. The findings suggest longer-term benefits for patients continuing treatment with pexidartinib. Outcomes for patients who discontinued pexidartinib should be evaluated in future studies.

Table 1. Summary of PGIC in physical function since initiating treatment with pexidartinib, worst stiffness NRS and worst pain NRS prior to and during pexidartinib treatment* in the baseline survey

Physical function (N=83)	N	%
Very much improved	25	30.1%
Much improved	24	28.9%
Minimally improved	16	19.3%
No change	14	16.9%
Minimally worse	1	1.2%
Much worse	1	1.2%
Very much worse	2	2.4%
Worst stiffness NRS (N=83)	**Mean**	**SD**
Score prior to pexidartinib treatment	6.2	2.8
Score during pexidartinib treatment	3.0	2.4
Change from prior to pexidartinib treatment	-3.2	3.1
Worst pain NRS (N=83)	**Mean**	**SD**
Score prior to pexidartinib treatment	5.7	2.7
Score during pexidartinib treatment	2.7	2.4
Change from prior to pexidartinib treatment	-3.0	2.7

PGIC, Patient Global Impression of Change.
* Patients were asked to recall their worst stiffness and worst pain prior to and during pexidartinib treatment.

Table 2. Patient reported outcomes, including PROMIS-PF, worst stiffness NRS, worst pain NRS, and TSQM scores in the baseline and follow up surveys

	Baseline	Follow Up	Change	P-value*
PROMIS-PF Overall				
N	31	27	27	
mean (SD)	44.29 (8.859)	43.06 (7.644)	-0.43 (5.724)	NS
Worst Stiffness Numeric Rating Scale (NRS)				
N	31	29	29	
mean (SD)	2.60 (2.610)	3.60 (2.460)	0.8 (2.67)	NS
Worst Pain Numeric Rating Scale (NRS)				
N	31	29	29	
mean (SD)	3.00 (2.890)	3.10 (2.750)	-0.1 (2.09)	NS
TSQM Effectiveness Domain Score				
N	31	29	29	
mean (SD)	68.64 (19.167)	73.75 (17.749)	7.28 (19.021)	0.04
TSQM Side Effects Domain Score				
N	31	29	29	
mean (SD)	60.89 (23.879)	59.27 (22.449)	0.86 (16.597)	NS
TSQM Convenience Domain Score				
N	31	29	29	
mean (SD)	65.05 (21.430)	63.98 (19.281)	1.34 (17.293)	NS
TSQM Global Satisfaction Domain Score				
N	31	29	29	
mean (SD)	69.12 (18.200)	71.18 (19.231)	4.19 (16.257)	NS

PROMIS-PF, Patient-Reported Outcomes Measurement Information System–Physical Function; TSQM, Treatment Satisfaction Questionnaire for Medication; SD, standard deviation; NS = Not Significant.
* P-value was from random slope regression model with time since first pexidartinib dose as independent variables.

Table 3. Summary of EQ-5D-5L in the follow up survey

Mobility (N=28)	N	%
I have no problems in walking about	9	32.1%
I have slight problems in walking about	11	39.3%
I have moderate problems in walking about	6	21.4%
I have severe problems in walking about	2	7.1%
I am unable to walk about	0	0.0%
Self-Care (N=29)		
I have no problems washing or dressing myself	20	69.0%
I have slight problems washing or dressing myself	6	20.7%
I have moderate problems washing or dressing myself	2	6.9%
I have severe problems washing or dressing myself	1	3.4%
I am unable to wash or dress myself	0	0.0%
Usual Activities (N=29)		
I have no problems doing my usual activities	12	41.4%
I have slight problems doing my usual activities	10	34.5%
I have moderate problems doing my usual activities	5	17.2%
I have severe problems doing my usual activities	2	6.9%
I am unable to do my usual activities	0	0.0%
Pain/Discomfort (N=29)		
I have no pain or discomfort	7	24.1%
I have slight pain or discomfort	15	51.7%
I have moderate pain or discomfort	4	13.8%
I have severe pain or discomfort	2	6.9%
I have extreme pain or discomfort	1	3.4%
Anxiety/Depression (N=29)		
I am not anxious or depressed	10	34.5%
I am slightly anxious or depressed	13	44.8%
I am moderately anxious or depressed	4	13.8%
I am severely anxious or depressed	2	6.9%
I am extremely anxious or depressed	0	0.0%

Table 4. Summary of PGIC in overall symptom since the start of the study in the follow up survey

Overall symptom (N=28)	N	%
Very much improved	4	14.3%
Much improved	15	53.6%
Minimally improved	5	17.9%
No change	4	14.3%
Minimally worse	0	0.0%
Much worse	0	0.0%
Very much worse	0	0.0%

PGIC, Patient Global Impression of Change.

(P 132) SARCOMA PATEINTS' VIEWS ON USING A RISK-PREDICTION MODEL TO INFORM TREATMENT DECISIONS.

Anouk A. Kruiswijk, Drs.[1]; Michiel van de Sande, MD, PhD[2]; Ellen G. Engelhardt, PhD[3]; Yvonne Schrage, MD, PhD[4]; Jos A. van der Hage, MD PhD[2]; Perla J. Marang-van de Mheen, PhD[5]; Leti van Bodegom-Vos, PhD[2]; VALUE- PERSARC research group, n/a[2], (1) Leiden University Medical Center, Amsterdam, Noord-Holland, Netherlands, (2) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (3) Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands, (4) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (5) Safety & Security Science, Faculty of Technology, Policy & Management, Delft University of Technology, Delft, Zuid-Holland, Netherlands

Objective: Risk prediction models (RPM) are statistical tools which can predict the likelihood of a certain outcome or event for an individual based on their (clinical) characteristics. RPM can support (shared) decision-making by providing patients and clinicians with insights into the diagnosis or prognosis of the disease. The number of RPM in medicine increased exponentially over the last decade, and also in the field of soft-tissue sarcoma (STS). Despite efforts to develop, validate and update RPM, it is unknown how sarcoma patients perceive and accept the use of RPM in their decision-making process. This study therefore evaluates the views of STS patients on using RPM to inform clinical decision-making.

Methods: A convergent parallel mixed-methods design is used, combining the results from a survey among 60 STS patients and semi-structured interviews among 10-15 STS patients treated in three tertiary sarcoma referral centers in the Netherlands. In these centers, the PERsonalised SARcoma Care (PERSARC) RPM was used by sarcoma specialists in multidisciplinary tumour boards to guide treatment advice and in patients consultations, where the oncological/orthopedic surgeon informs the patient about his/her diagnosis and discusses benefits and harms of all relevant treatment options. Specifically for the latter reason, the PERSARC RPM was implemented in a new patient-friendly app called VALUE-PERSARC.

Results: Preliminary results based on 6 interviews with STS patients and 28 completed questionnaires showed that 89% of the patients perceived the PERSARC, embedded in the VALUE-PERSARC app as useful and the majority (88%) would use VALUE-PERSARC again. Only one-third (32%) reported that the RPM aided in clinical decision-making. The most mentioned reasons for STS patients to promote VALUE-PERSARC was that it helped in understanding the impact of different treatment options (25%) and provided insights into personalized risk estimates (21%). Interviews revealed that the RPM clarified the information provided by the clinician, but not per se to support (shared) decision-making often because only

one treatment option was discussed with the PERSARC RPM.

Conclusion: Patients perceived the use of VALUE-PERSARC as helpful to support information provision but it did not facilitate (shared) decision-making, mostly because patients did not feel there was a choice in treatment. Our findings highlights the importance of studies investigating the influence of RPM in daily clinical practice, to evaluate if it worked as intended to actually support (shared) decision-making. Future research should explore how to best involve patients in their decision-making process when using a RPM.

(P 133) TEN RECOMMENDATIONTS FOR SARCOMA SURGERY: CONSENSUS OF THE SURGICAL SOCIETIES BASED ON THE GERMAN S3 GUIDELINE "ADULT SOFT TISSUE SARCOMA"

Jens Jakob, MD[1]; Dimosthenis Andreou, MD[2]; Jens Bedke, MD[3]; Dominik Denschlag, MD[4]; Hans Roland Dürr, MD[5]; Thomas Gösling, MD[6]; Thomas Graeter, MD[7]; Viktor Grünwald, MD[8]; Robert Grützmann, MD[9]; jürgen Hoffmann, MD[10]; Ingolf Juhazs-Boess, MD[11]; Bernd Kasper, MD, PhD[12]; Vlada Kogosov, PhD[13]; Wolfram Trudo Knoefel, MD[14]; Burkhard Lehner, MD[10]; Marcus Lehnhardt, MD[15]; Lars H. Lindner, MD[16]; Cordula Matthies, MD[17]; Jalid Sehouli, MD[18]; Selma Ugurel, MD[8]; Peter Hohenberger, N/A, n/a[19], (1) University Medical Center Mannheim, University of Heidelberg, Mannheim, Baden-Wurttemberg, Germany, (2) Medical University of Graz, Graz, Steiermark, Austria, (3) Klinikum Stuttgart, Stuttgart, Baden-Wurttemberg, Germany, (4) Hochtaunus Klinik, Bad Homburg v.d.H, Hessen, Germany, (5) Orthopaedic Oncology, Department of Orthopaedics and Trauma Surgery, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (6) Klinikum Braunschweig, Braunschweig, Niedersachsen, Germany, (7) SLK-Lungenklinik Lowenstein, Loewenstein, Baden-Wurttemberg, Germany, (8) University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (9) Department of General and Visceral Surgery, University Hospital, Friedrich-Alexander- University Erlangen-Nuremberg, 91054 Erlangen, Germany, Erlangen, Bayern, Germany, (10) University Hospital Heidelberg, Heidelberg, Baden-Wurttemberg, Germany, (11) University Hospital Freiburg, Freiburg, Baden-Wurttemberg, Germany, (12) University of Heidelberg, Mannheim University Medical Center, Mannheim, Baden-Wurttemberg, Germany, (13) Comprehensiv Cancer Center Lower Saxony, Göttingen, Niedersachsen, Germany, (14) University Hospital Duesseldorf, Düsseldorf, Nordrhein-Westfalen, Germany, (15) BG University Hospital Bergmannsheil, Bochum, Nordrhein-Westfalen, Germany, (16) Department of Medicine III, LMU University Hospital, LMU Munich, Munich, Bayern, Germany, (17) University Hospital Wuerzburg, Würzburg, Bayern, Germany, (18) Charité, Berlin, Berlin, Germany, (19) Sarcoma Unit, Department of Surgery and Mannheim Cancer Center, University Medical Center Mannheim, University of Heidelberg, Mannheim,Germany, Mannheim, Baden-Wurttemberg, Germany

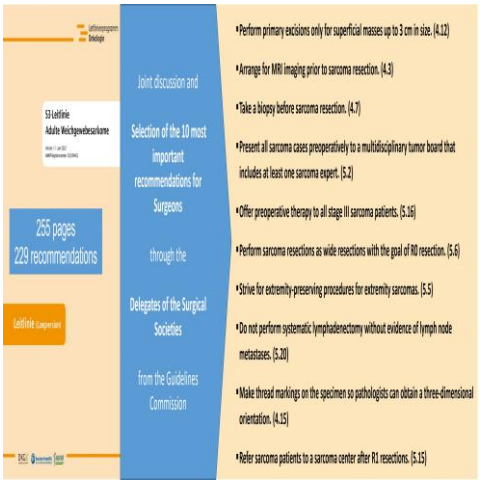
Objective: Surgeons of any specialty are often the first point of contact for sarcoma patients. Due to limited experience and knowledge of the existing guidelines, the initial diagnosis and therapy are error-prone in many cases. We selected the TOP TEN recommendations of sarcoma surgery for surgeons by surgeons in a Delphi process to improve the distribution of the guideline "Adult Soft Tissue Sarcomas" (AWMF Registry No. 032/044OL).

Methods: A Delphi process was applied. The Delegates from the surgical societies that were involved in the guideline process selected the 15 recommendations that were most important to

them. Votes for similar recommendations were added together. From the resulting ranking, the 10 recommendations with the most votes were selected and confirmed by consensus in the next step.

Results: The statement "Resection of primary soft tissue sarcomas of the extremities should be performed as a wide resection. The goal is an R0 resection" was selected as the most important term. The next highest ranked recommendations were the need for a preoperative biopsy, performing preoperative MRI imaging with contrast, and discussing all cases before surgery in a multidisciplinary sarcoma committee.

Conclusion: The development and adoption of the evidence-based guideline "adult soft tissue sarcomas" was a milestone in SArcoma therapy in Germany. The present project with a selection of a "TOP TEN" list of recommendations for primary care physicians by the delegates of their own societies serves the dissemination of the most important recommendations of the guideline.



(P 134) IMPACT OF THE PARTNERSHIP BETWEEN TERTIARY CENTERS AND NON-SARCOMA RADIOLOGY CENTERS.

Sophie Mottard, Dr[1]; Georges Basile, Dr[2]; Hugo Saint-Yves, M.Sc[3]; Isabelle Drapeau, Dr[3]; Marie-Josée Berthiaume, Dr[3]; Alexandra Auger[3]; Marc Isler, Dr[3]; Janie Barry, MSc[3], (1) CIUSSS Est-de-l'Île-de-Montréal, montreal, PQ, Canada, (2) University of Montreal, Montreal, PQ, Canada, (3) CIUSSS Est-de-l'Île-de-Montréal, Montreal, PQ, Canada

Objective: Sarcomas are aggressive lesions requiring early management by a specialized multidisciplinary team. Due to their great variability in terms of affected tissue, location, size and evolution, their detection remains challenging. As part of the development of the Quebec Sarcoma Network, new referral algorithms have been established to facilitate the referral of patients to tertiary sarcoma treatment centers. As described in a previous study, the time between the first imaging and patient referral constituted around 37% of the total time between the onset of symptoms and the first consultation in a specialized center (mean delay of 66 weeks (median 22)). It was also observed that 60% of patients had to undergo an additional imaging investigation in a sarcoma center, due to insufficient or obsolete imaging. A plan to

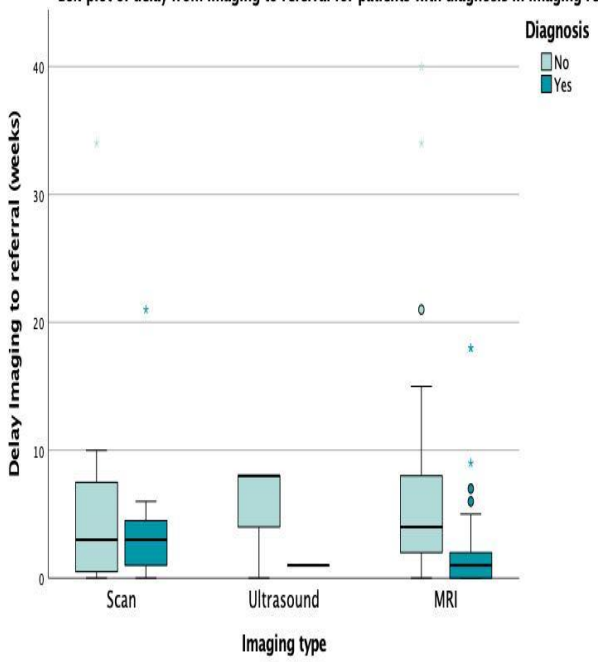
optimize the partnership with radiologists not specialized in sarcomas was therefore put in place. This study presents preliminary results of this partnership impact on the sarcoma care pathway.

Methods: This is a retrospective study of 100 consecutive consultations requested between October 14, 2022 and May 9, 2023. Local ERB approbation was obtained. The following exclusion criteria were applied: non-oncological cases, bone metastases, superficial lesions of less than 5 cm. Patients still under investigation were also excluded. Referral data was collected from medical records: type of and date of examination, final diagnosis, grade (categorized as benign or malignant), subtype (soft tissue or bone) and date of referral to a sarcoma center. Imaging reports were assessed whether or not a diagnosis was mentioned, and if a referral to a specialized sarcoma center was recommended. Time from imaging to referral is given as the mean number of weeks (min-max; SD), and other findings as frequency and percentages. The Man Whitney statistical test was used to compare delays for categorical data, to account for the non-normality of the data. Analyses comparing categorical data were performed using the chi-square test.

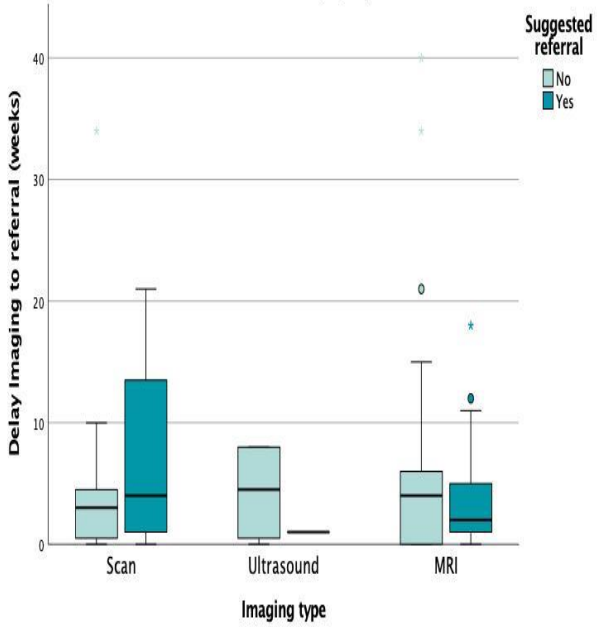
Results: There were 69 soft-tissue lesions (33 (48%) malignant, 36 (52%) benign) and 31 bone lesions (6 (20%) malignant, 25 (80%) benign) in the reviewed cohort. 78 patients had an MRI (74% for a soft-tissue lesion), of whom 35 (45%) included a specific diagnosis linked to a consultation in a sarcoma center, and 42 (54%) suggested a referral to a sarcoma center in the imaging report. Ultrasounds were performed for 52 patients, of whom 5 (10%) included a specific diagnosis and 1 (2%) suggested a referral to a sarcoma center. 35 patients (69% soft tissue) had a CT scan, of whom 13 (37%) had a specific diagnosis and 9 (26%) suggested a referral to a sarcoma center. Delay from imaging to referral for patients with a specific diagnosis in imaging report was 3 weeks (0-21; 4.9) versus 6.9 weeks (0-40; 8.9) ($p=0.003$) for those who did not have this information. Mean time for cases where the imaging report suggested referral to a sarcoma center was 3.8 weeks (0-21; 5.0) versus 6.6 (0-40; 9.3) ($p=0.268$) for those who did not receive this recommendation.

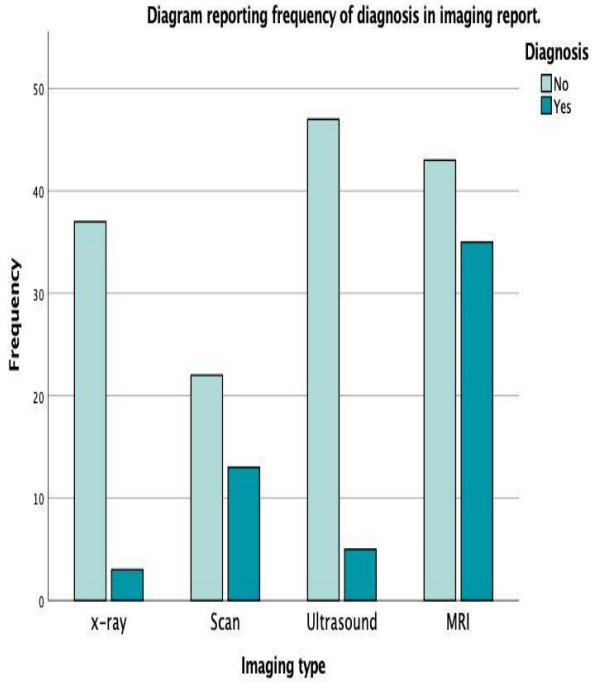
Conclusion: The radiologists' impact in the sarcoma diagnostic pathway is major. Incorporating an accurate diagnosis in imaging reports enables doctors and specialists to refer patients more efficiently to sarcoma centers. Clear referral recommendation to a specialized sarcoma center also helps to reduce referral times, although the results are not statistically significant. Optimizing radiologists' training, both on the sarcoma suspicion and on the importance of referring physicians to the appropriate resources, will significantly improve the sarcoma care pathway. Collaboration with radiologists could be achieved through teaching, institutional presentations, webinars, conference presentations - in short, make some noise!

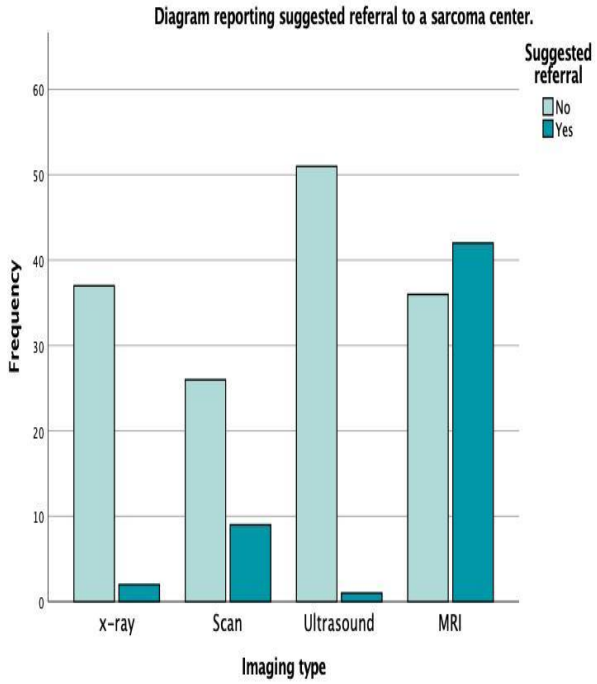
Box plot of delay from imaging to referral for patients with diagnosis in imaging report.



Box plot of delay from imaging to referral for patients with suggested referral to a sarcoma center in imaging report.




Diagram reporting frequency of diagnosis in imaging report.
Diagnosis
No
Yes
Frequency
50
40
30
20
10
0
x-ray
Scan
Ultrasound
MRI
Imaging type


Diagram reporting suggested referral to a sarcoma center.
Suggested referral
No
Yes
Frequency
60
50
40
30
20
10
0
x-ray
Scan
Ultrasound
MRI
Imaging type

(P 135) UNMET NEEDS IN LOCALLY ADVANCED (UNRESECTABLE) OR METASTATIC DEDIFFERENTIATED LIPOSARCOMA AND EXPECTATIONS FOR CLINICAL TRIALS: AN INTERNATIONAL DELPHI CONSENSUS STUDY

Patrick Schöffski, MD, MPH[1]; Mark Agulnik, MD[2]; Jean-Yves Blay, MD, PhD[3]; Anna Chalmers, MD[4]; Antoine Italiano, MD,PhD[5]; Robin L. Jones, BSc, MB BS, MRCP, MD[6]; Daniel Pink, MD[7]; Silvia Stacchiotti, MD[8]; Claudia Valverde, MD[9]; Bruno Vincenzi, MD, PhD[10]; Michael J. Wagner, MD[11]; Robert G. Maki, MD PhD[12], (1) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium, (2) City of Hope, Department of Medical Oncology & Therapeutics Research, Duarte, CA, (3) Centre Léon-Bérard, Lyon, Rhone-Alpes, France, (4) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, (5) Institut Bergonié, Bordeaux, Aquitaine, France, (6) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (7) Helios Hospital Bad Saarow, Sarcoma Centre Berlin-Brandenburg, Department of Hematology, Oncology and Palliative Care, Bad Saarow, Brandenburg, Germany, (8) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (9) Vall D'Hebron University Hospital, Barcelona, Catalonia, Spain, (10) Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Lazio, Italy, (11) University of Washington/Fred Hutch/Seattle Cancer Care Alliance, Seattle, WA, (12) Memorial Sloan Kettering Cancer Center; Professor, Weill Cornell Medical College, New York, NY

Objective: Locally advanced (unresectable) or metastatic dedifferentiated liposarcoma (DDLPS) is a common subtype of liposarcoma. It is often high-grade and aggressive and most often occurs in the retroperitoneum and deep soft tissue of proximal extremities. Anthracycline-based therapy, such as doxorubicin, is considered the standard of care in first line (1L) treatment of DDLPS, either used as a single agent or in combination with an alkylating agent. The objective of this study was to gather expert opinions across Europe and the US on DDLPS management and unmet needs, Progression Free Survival (PFS) relevance as a clinical trial endpoint and expectations for clinical trial design. The study also aimed at building robust evidence on key clinical topics to raise awareness among the broader scientific community and educate key stakeholders across healthcare systems.

Methods: A modified Delphi panel study was conducted to assess and fill gaps in key clinical topics in 1L DDLPS. We recruited a panel of sarcoma experts (N=12) with expertise in treating DDLPS and clinical trial design across Europe (France, Germany, Italy, Spain, UK, and Belgium) and the US. The study consisted of two rounds of anonymous surveys with pre-defined statements covering key questions. Surveys also included a request for feedback, which served to modify the statements on which consensus was not reached. Subsequently, revised statements were discussed in an unblinded consensus meeting to allow panel discussions, which resulted in a final list of agreed-upon statements.

Results: We tested 55 statements across different topics related to 1L DDLPS, including disease burden, treatment paradigm, unmet needs, PFS relevance as an endpoint, and cross-over trial design; consensus was reached on 43 statements. Experts agreed on 8 statements on disease burden, noting symptoms such as discomfort, abdominal distension, and fatigue among the most frequently reported. Experts reached consensus on 8 statements on treatment paradigm, agreeing that doxorubicin is the standard of care but acknowledging they are not fully satisfied with the treatment due to inadequate efficacy and frequent adverse events. Consensus was reached on 7 statements on unmet needs, highlighting diagnostic and referral

challenges and the poor efficacy and safety of current treatments. Experts reached consensus on 15 statements on the relevance of PFS, agreeing that it is frequently used as a primary endpoint and that it is both clinically and patient relevant in 1L DDLPS. Lastly, experts agreed on 5 statements on the cross-over trial design, noting its ethical benefits and ability to adjust for cross-over effects in Overall Survival (OS) analyses. There were no statements where experts collectively disagreed.

Conclusion: This study shows that there is strong consensus around the high disease burden of DDLPS, which often leads to deterioration of patients' quality of life. Experts agreed on the need for treatments with a better clinical profile, as they are not fully satisfied with the efficacy and safety of 1L treatments, including doxorubicin. Regarding the relevance of PFS, experts concluded that it is a direct measure of treatment effect and a good surrogate endpoint for survival benefit in 1L DDPLS, and therefore, that there is a clinically meaningful relationship between PFS and OS. On the cross-over clinical trial design, experts concluded it is well-accepted because it can encourage patient participation. Ultimately, there was alignment on key clinical endpoints and cross-over trial design being deemed appropriate and acceptable in 1L DDLPS. This study indicates that there is an alignment across geographies regarding the unmet need and treatment of DDLPS, and expectations for clinical trials. Raising awareness of DDLPS management and unmet needs, as well as the value of PFS and a cross-over clinical trial design, can contribute to improving patient outcomes and supporting the development of innovative treatments.

(P 136) SETTING THE INTERNATIONAL RESEARCH AGENDA FOR SARCOMA WITH PATIENTS AND CARERS: PRELIMINARY RESULTS OF PHASE II OF THE SARCOMA PATIENT ADVOCACY GLOBAL NETWORK (SPAGN) PRIORITY SETTING PARTNERSHIP

Evelyne Roets, MD[1]; Olga Husson, PhD[1]; Sorrel R B Bickley, PhD[2]; Markus Wartenberg[3]; Ornella Gonzato, Dr.[4]; Natalia Fernandez, PhD[5]; Bernd Kasper, MD, PhD[6]; Kai Pilgermann[7]; Roger Wilson, CBE HonMD HonLLD[8]; Neeltje Steeghs, Dr.[9]; Winette van der Graaf, Prof. Dr.[9]; Gerard Van Oortmerssen, Dr.[10]; Kathrin Schuster[11], (1) Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands, (2) Sarcoma UK, London, England, United Kingdom, (3) German Sarcoma Foundation (DSS), SPAGN, Woelfersheim, Hessen, Germany, (4) Sarcoma Patient Advocacy Global Network Assoc. (SPAGN), Udine, Friuli-Venezia Giulia, Italy, (5) SPAGN Sarcoma Patient Advocacy Global Network, Barcelona, Catalonia, Spain, (6) University of Heidelberg, Mannheim University Medical Center, Mannheim, Baden-Wurttemberg, Germany, (7) Sarcoma Patient Advocacy Global Network (SPAGN), Wölfersheim, Hessen, Germany, (8) Founder - Sarcoma UK, London, England, United Kingdom, (9) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (10) Sarcoma Patient Advocacy Global Network Assoc. (SPAGN), Woelfersheim, Hessen, Germany, (11) Sarcoma Patient Advocacy Global Network (SPAGN), Woelfersheim, Hessen, Germany

Objective: Typically, researchers and clinicians determine the agenda in sarcoma research. However, patient involvement can have a meaningful impact on research. Therefore, the Patient-Powered Research Network (PPRN) of the Sarcoma Patient Advocacy Global Network (SPAGN) set up a Priority Setting Partnership (PSP). The primary objective of this study is to identify priorities for research and patient advocacy topics.

Methods: During the first phase of the study, a collaboration was set up between SPAGN and

stakeholders from the sarcoma field. This study included patients and survivors of sarcoma (including GIST and desmoid fibromatosis), carers or family members of people who have (had) sarcoma and patient advocates. In the first phase of this study, unanswered questions regarding sarcomas and topics for patient advocacy groups were identified using an online questionnaire. Results of the first phase of the study were refined to a list of 24 research topics (table 1) and 14 patient advocacy topics (table 2). Research topics focus on the origin of sarcoma, the diagnostic process, treatment and side effects, prognosis, quality of life and end of life. Topics for patient advocacy focus on the diagnostic process, communication between patients and caregivers, data sharing, information, quality of life, end of life, expert centers and off-label or compassionate use medications. In the second phase of the online survey, participants are asked to fill in a top 5 and a top 3 of research and patient advocacy topics, respectively. The following sociodemographic characteristics are collected: gender, age, respondent's connection with sarcoma (e.g. patient, carer), ethnic origin, country of residence and educational level of the respondent. For sarcoma characteristics, the following items were collected: tumor type and location, intention of treatment (e.g. curative, palliative), type of treatment, disease stage and current situation (e.g. active treatment, follow-up). Awareness for the survey is being created via the network of SPAGN and social media.

Results: The first phase of the survey resulted in 264 participants (ESMO Open 2022). A preliminary analysis in May 2023 showed that 408 patients, carers and patient advocates filled out the second part of the survey. The majority of participants are patients diagnosed with a soft tissue sarcoma, GIST or bone sarcoma. Currently included participants are living in Europe, the United States, Asia and Brazil.

Conclusion: The online survey will be open for participation until July 2023. Results will be presented at CTOS 2023. We aim to have an accurate representation of the sarcoma community involving patients with diverse sarcoma subtypes and demographic backgrounds. These results could provide guidance for investigators and can assist in improving sarcoma patient's care.

Table 1 Subjects for research.

1	What are causes of sarcoma?
2	Are there ways to prevent sarcoma?
3	Can vaccines be developed to prevent or treat sarcomas?
4	In which way are hereditary aspects involved in the development of sarcoma?
5	More research specific on subtypes of sarcoma (eg. GIST, retroperitoneal liposarcoma, angiosarcoma,...) is needed.
6	Which personal characteristics have sarcoma survivors in common (think of psychological, medical and sociodemographic characteristics)?
7	What are the most accurate techniques for the diagnosis of sarcoma (think of imaging modalities, blood tests, whole genome sequencing, etc) and which techniques or strategies could be used to improve the distinction between different subtypes of sarcoma and between benign and malignant tumors?
8	What percentage of people with sarcoma receive the wrong diagnosis in the first instance?
9	What is the risk of taking a biopsy?
10	What is the role of immunotherapy, targeted therapy and combined therapy in the treatment of sarcomas?
11	What is the effect of different treatment modalities on survival and quality of life?
12	More research is needed into novel surgery techniques.
13	What is the effect of different surgical techniques and surgical margins on the outcome for the patient (think of functional outcomes, prognosis, recurrence, etc)?
14	What is the effect of lifestyle (diet, physical activity, etc) on the development of sarcoma?
15	What is the effect of lifestyle on the outcome (e.g. quality of life) during and after treatment?
16	What are the possible treatment methods (e.g. psychotherapy, mindfulness, psychedelics) for disease-related mental suffering (e.g. acceptance, anxiety)?
17	What are the side effects of the different treatment options (targeted therapy, chemotherapy, radiotherapy, surgery, etc.) and how can these side effects be treated?
18	What are the long term effects of sarcoma treatment on intimacy and fertility?
19	What is the prognosis and the risk of recurrence of sarcoma and which factors have an effect on this?
20	How can follow-up scheme for sarcoma patients be better personalized?
21	How can the re-integration of sarcoma survivors in the society be facilitated (think of work re-integration, social re-integration)?
22	How is end-of-life care organised (in different countries)?
23	What is happening in the terminal phase (development of the disease) and what are the best methods to give best supportive care?
24	What is the role of carers in the final phase of life and how can carers support the patient in taking decisions in the final phase of life?

Table 2 subjects for patient advocacy

1	Improving the diagnostic process of sarcoma through better education and development of tools that can assist general practitioners in recognizing the possibility of a sarcoma.
2	A better classification is needed for benign and malignant tumors. Benign tumors should be included in tumor registries.
3	Analysis of the tumor DNA should be available for all patients.
4	Data sharing should be improved; all relevant data of a patient should be available across medical centers.
5	An international registry with data about sarcoma patients is needed to supply data for research and stimulate international research collaboration.
6	Communication between specialists and patient must be improved to stimulate shared decision-making.
7	A single point of contact must be provided to patients (e.g. case manager, specialized nurse).
8	Information on all tumor subtypes must be available for patients.
9	Sarcoma centers should advise patients on complementary treatments, lifestyle and diet.
10	More attention should be given to quality of life and consequences of treatment (e.g. pain, temporary/permanent effects of surgery, side effects of medication) during the shared-decision making process.
11	Mental support must be available for sarcoma patients.
12	End-of-life scenario should be discussed openly and timely with the patient.
13	Referral of patients to sarcoma expert centers, centralization, networks.
14	The availability to patients of off-label or compassionate use medication.

(P 137) **IMPACT OF SOCIAL DETERMINANTS OF HEALTH ON SARCOMA CARE: A SINGLE INSTITUTION EXPERIENCE**

Emily M. Peairs, BS[1]; Kiera Lunn, BS[2]; Mikhail A. Bethell, MS[1]; Sharrieff N. Shah, BS[1]; Alexandra Krez, BA[2]; Elizabeth Sachs, MS[3]; Chad E. Cook, PhD[4]; Brian Brigman, MD, PhD[3]; William Eward, MD, DVM[3]; Julia Visgauss, MD[3], (1) Duke University School of Medicine, Durham, NC, (2) Duke School of Medicine, Durham, NC, (3) Duke University, Durham, NC, (4) Duke University, DURHAM, NC

Objective: Previous research has shown that sarcoma patients treated at experienced, high-volume centers have better outcomes. However, social and demographic factors may present barriers to accessing these centers. This study aims to identify social determinants of health (SDoH) factors that affect sarcoma treatment adherence and oncologic outcomes.

Methods: Patients diagnosed with sarcoma at a single institution between 2016 and 2021 were surveyed about SDoH factors and demographic characteristics. Survey questions were derived from previously validated surveys, and data was collected from medical charts regarding oncologic outcomes, treatment adherence, and complications. Descriptive statistics and correlation matrices were used to analyze the data.

Results: Of the 70 patients who completed the survey, 66 had at least one social risk factor (94.29%). The most commonly reported SDoH category was social and community context (83.3% of respondents), followed by healthcare access (68.1%), economic stability (26.4%), and neighborhood and built environment (12.5%). Weak but significant correlations existed between health care access scores and time to treatment, high area deprivation index and metastasis, and income and missed appointments (Table 1 and 2).

Conclusion: Our study highlights the prevalence of social risk factors among sarcoma patients and underscores the importance of addressing them in clinical settings to improve treatment adherence and outcomes. We found that social and community context and health care access were significantly associated with sarcoma treatment outcomes, indicating that interventions targeting these factors may improve patient outcomes.

Table 1: Demographic Characteristics of Survey Respondents

	N = 70
Average age	55.6 years
Female sex	51.4%
Race/ethnicity	
White	84.3%
Black/African American	7.1%
Hispanic	4.3%
Asian	2.9%
In-state Residents	61.4%
Insurance type	
Private	51.4%
Medicaid	4.3%
Medicare	31.4%
Other	13%
Highest education level completed	
High school	20%
College	50%
Graduate/Post-graduate	27.1%
Own the home they live in?	84.5%
Work status	
Full-time	41.4%
Part-time	8.6%
Other (retired, unemployed)	50%
Income level	
Under $20,000	5.7%
$20,000 - $40,000	5.7%
$40,000 - $63,000	12.9%
Over $63,000	64.3%
Choose not to answer	11.4%

Table 2: Relationship between SDoH Scoring and Clinical Management Variables

	Total SDoH Score	Economic Stability	Health Care Access	Neighborhood and Physical Environment	Social and Community Context
Time to treatment (days)	-.227 (p=0.06)	-.071 (p=0.57)	-.257 **(p=0.03)**	-.112 (p=0.37)	-.169 (p=0.17)
On/Off Protocol	-.019 (p=0.87)	-.045 (p=0.71)	-.058 (p=0.63)	-.138 (p=0.25)	.017 (p=0.89)
Number of Missed/Cancelled Orthopaedic Appointments	.032 (p=0.79)	.058 (p=0.63)	-.095 (p=0.43)	-.162 (p=0.17)	-.025 (p=0.84)
Number of Cancelled Ancillary Appointments	-.121 (p=0.31)	-.122 (p=0.30)	-.229 (p=0.05)	-.131 (p=0.27)	-.111 (p=0.35)
Received Neoadjuvant or Adjuvant Radiation at a Local Facility (Y/N)	.215 (p=0.07)	.196 (p=0.10)	.209 (p=0.08)	.219 (p=0.06)	.234 **(p=0.04)**

For medically based comparisons, correlation coefficient interpretation boundaries of Zero=none, 0.1 to 0.2=poor, 0.3 to 0.5=fair, 0.6 to 0.7= moderate, and 0.8 to 0.9 very strong, have been suggested.

Table 3: Demographic factors and Oncologic Outcomes

	Non-White	Hispanic	Education	Insurance Status	Income	ADI of 6 or Greater
Size at diagnosis	.012 (p=0.93)	.006 (p=0.96)	-.099 (p=0.44)	.106 (p=0.39)	.114 (p=0.36)	-.033 (p=0.79)
Metastasis	.161 (p=0.19)	.103 (p=0.39)	-.040 (p=0.74)	.016 (p=0.89)	-.083 (p=0.49)	.242 **(p=0.04)**
Local Recurrence	-.006 (p=0.96)	-.086 (p=0.48)	-.092 (p=0.44)	.140 (p=0.24)	-.102 (p=0.39)	.180 (p=0.13)
Complications	.037 (p=0.77)	-.157 (p=0.19)	-.194 (p=0.11)	.027 (p=0.82)	-.049 (p=0.68)	.063 (p=0.59)
Number of Missed/Cancelled Orthopaedic Appointments	-.109 (p=0.37)	.010 (p=0.94)	-.203 (p=0.09)	.149 (p=0.21)	.249 **(p=0.04)**	.109 (p=0.36)
Number of Cancelled Ancillary Appointments	-.107 (p=0.38)	.258 **(p=0.03)**	-.018 (p=0.89)	-.024 (p=0.84)	-.138 (p=0.28)	-.031 (p=0.79)

For medically based comparisons, correlation coefficient interpretation boundaries of Zero=none, 0.1 to 0.2=poor, 0.3 to 0.5=fair, 0.6 to 0.7= moderate, and 0.8 to 0.9 very strong, have been suggested.
ADI refers to Area Deprivation Index, a measure of the average SES based on home address.

(P 138) CONTROVERSIES IN THE MANAGEMENT OF PATIENTS WITH SOFT TISSUE SARCOMA: RESULTS AND RECOMMENDATIONS FROM THE FIRST CONFERENCE ON STATE OF SCIENCE IN SARCOMA (CSSS)

Dimosthenis Andreou, MD[1]; Silvia Hofer, MD[2]; Jean-Yves Blay, MD, PhD[3]; Thomas Brodowicz, MD[4]; Ingrid M. Desar, MD PhD[5]; Palma Dileo, MD[6]; Hans Gelderblom, MD, PhD[7]; Rick Haas, MD, PhD[8]; Jens Jakob, MD[9]; Robin L. Jones, BSc, MB BS, MRCP, MD[10]; Ian R. Judson, MA, MD, FRCP[11]; Wolfgang G. Kunz, MD[12]; Andreas Leithner, Prof. MD[1]; Bernadette Liegl-Atzwanger, Prof. MD[1]; Lars H. Lindner, MD[13]; Christina Messiou, MRCP, FRCP. MD[14]; Aisha B. Miah, MD[14]; Peter Reichardt, MD, PhD[15]; Joanna Szkandera, MD[1]; Winette van der Graaf, Prof. Dr.[16]; Winan J. van Houdt, MD, PhD, MS[16]; Eva Wardelmann, MD[17]; Christian Rothermundt, MD[18], (1) Medical University of Graz, Graz, Steiermark, Austria, (2) University Hospital Zurich, Zurich, Zurich, Switzerland, (3) Centre Léon-Bérard, Lyon, Rhone-Alpes, France, (4) Medical University of Vienna, Vienna, Wien, Austria, (5) Radboud University Medical Center, Nijmegen, Gelderland, Netherlands, (6) University College Hospital, London, England, United Kingdom, (7) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (8) The Netherlands Cancer Institute, Amsterdam, Drenthe, Netherlands, (9) University Medical Center Mannheim, University of Heidelberg, Mannheim, Baden-Wurttemberg, Germany, (10) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (11) Institute of Cancer Research, London, England, United Kingdom, (12) Department of Radiology, University Hospital, Ludwig-Maximilians-University Munich, 81377 Munich, Germany, Munich, Bayern, Germany, (13) Department of Medicine III, LMU University Hospital, LMU Munich, Munich, Bayern, Germany, (14) The Royal Marsden Hospital, London, England, United Kingdom, (15) Helios Klinikum Berlin-Buch and Medical School Berlin, Berlin, Berlin, Germany, (16) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (17) Institute of Pathology,.University Hospital Muenster, Muenster, Nordrhein-Westfalen, Germany, (18) Cantonal Hospital St. Gallen, St. Gallen, Sankt Gallen, Switzerland

Objective: International guidelines on soft tissue sarcoma (STS) cover primarily aspects, for which good quality evidence exists. However, they do not cover controversial topics discussed among sarcoma experts in the absence of prospective data or in case of conflicting results from small retrospective or low-quality studies. Consensus expert recommendations are therefore needed to complement these guidelines.

Methods: We organized a consensus meeting of 62 European sarcoma experts from 12 countries, covering different specialities involved in research and management of patients with STS. We used a modified Delphi process to identify and evaluate 220 controversial questions regarding STS diagnostics, peri-operative, local, and palliative treatment, follow-up, trial design, and politics. A recommendation agreed upon by >=75% panelists was defined as a “consensus”, while an agreement by >=95% of the panelists was characterized as “strong consensus”. The process was designed to allow the identification of areas without consensus, where additional data collection or future research was deemed necessary.

Results: A consensus could be reached in 30 topics, such as recommending a core-needle rather than incisional biopsy for histological diagnosis, and preoperative rather than postoperative chemotherapy in patients with extremity or trunk wall STS. There was also consensus in the preferred treatment of patients with metachronous oligometastatic disease involving both local and systemic treatments, and in recommending against reimbursement of

STS surgery outside reference centers. The panelists also consented that the radiographic features of STS should be considered when estimating STS grade, especially if tumor grading is not conclusive on biopsy, and that quality of life, socioeconomic (including insurance issues), and psychological aspects should be systematically and longitudinally assessed in daily clinical practice. There was strong consensus in additional 16 topics, such as basing surgical strategies on both tumor characteristics and surgical morbidity/function preservation, recommending randomized discontinuation trials in palliative patients with slowly growing STS achieving stable-disease, including all patients with newly recognized molecular entities in international registries and requiring a a combined response score from pathology and radiology in future trials. On the other hand, no consensus was achieved on how high-risk STS or the adolescent and young adult (AYA) population should be defined, the goals of surgical treatment in patients with progressive disease under preoperative treatment, or the optimal time interval between preoperative radiotherapy and subsequent surgery. There was also no consensus on the treatment sequence for high-risk extremity or trunk wall STS, on treatment recommendations following an unplanned R1/R2 excision (whoops resection) with or without macroscopic tumor left, on when next-generation-sequencing should be performed in the palliative setting or on the optimal endpoints for future trials in advanced/metastatic STS.

Conclusion: Several consensus recommendations could be identified, however they comprised only 21% of the available topics. For a variety of sarcoma management aspects, future research questions could be formulated, whereas for some topics, it appears unlikely that there will ever be sufficient data or a consensus.

(P 139) ULTRA-RARE SARCOMAS HAVE WORSE SURVIVAL COMPARED TO NON-ULTRA-RARE SARCOMAS: A NATIONAL CANCER REGISTRY STUDY

Chia-Chen Li, MD[1]; Ruru Chun-Ju Chiang, PD D[2]; Ming-Jing Lee, MD[3]; Ya-Wen Yang, n/a[2]; Bo-Zhi Zhuang, n/a[2]; Wen-Chung Lee, PD D[2]; Tom Wei-Wu W. Chen, MD, PhD[4], (1) National Taiwan University Cancer Center, Taipei, Taipei, Taiwan (Republic of China), (2) National Taiwan University, Taipei, Taipei, Taiwan (Republic of China), (3) National Taiwan University Hospital Hsin-Chu Branch, Taipai, Taipei, Taiwan (Republic of China), (4) National Taiwan University Hospital, Taipei, Taipei, Taiwan (Republic of China)

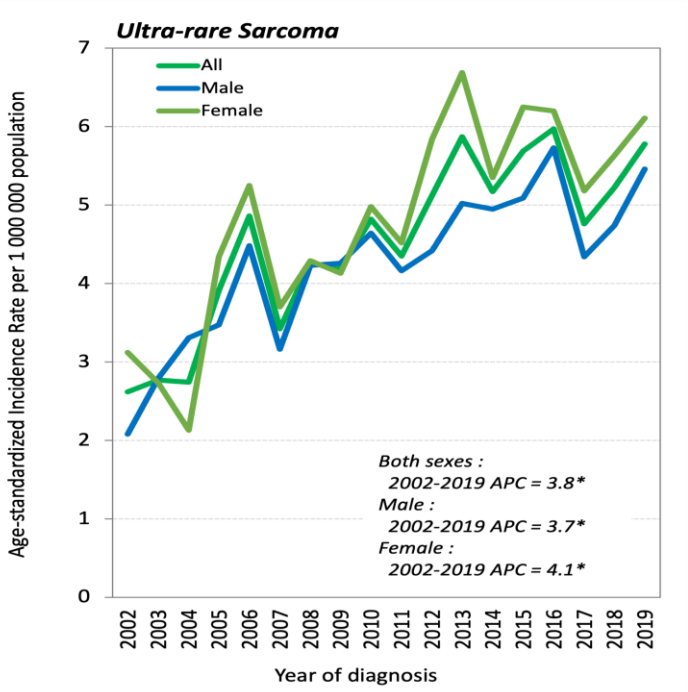
Objective: Our understanding of the clinical characteristics, survival rate, and prognostic factors of ultra-rare sarcomas (URSs) is limited due to the low incidence. The aim of this study is to understand the clinical differences among URS, non-URS (NURS), and other non-sarcoma solid tumors (NSST).

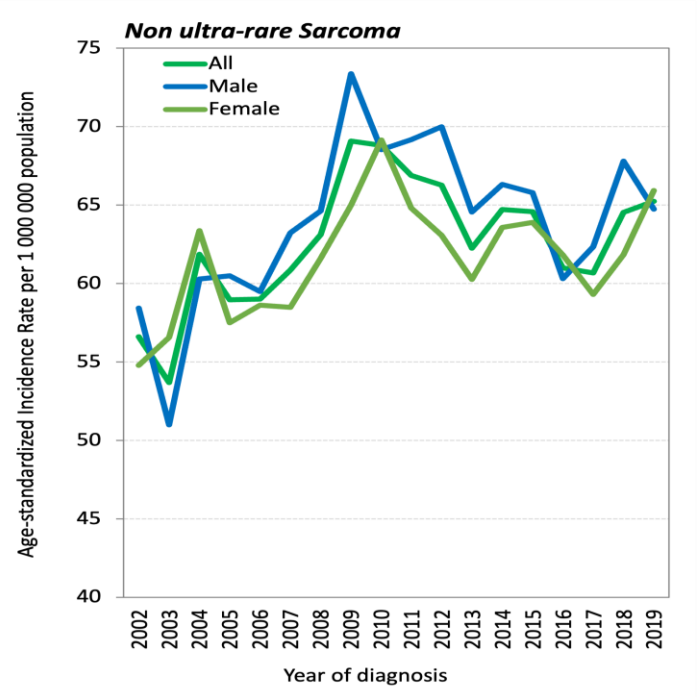
Methods: Clinical demographics and survival of solid tumor patients(pts) of Taiwan Cancer Registry diagnosed between January 1, 2002, and December 31, 2019, were retrieved. URS included sarcoma histologies with an incidence < 1/1,000,000 persons. The annual percent change (APC) was computed to summarize the change in incidence for the recent years. Overall survival (OS) was estimated from the time of diagnosis to death or the last follow-up. The impact of gender, age, diagnosis period (2002-2010 vs. 2011-2019), and histology type on OS were estimated by Cox proportional hazard method.

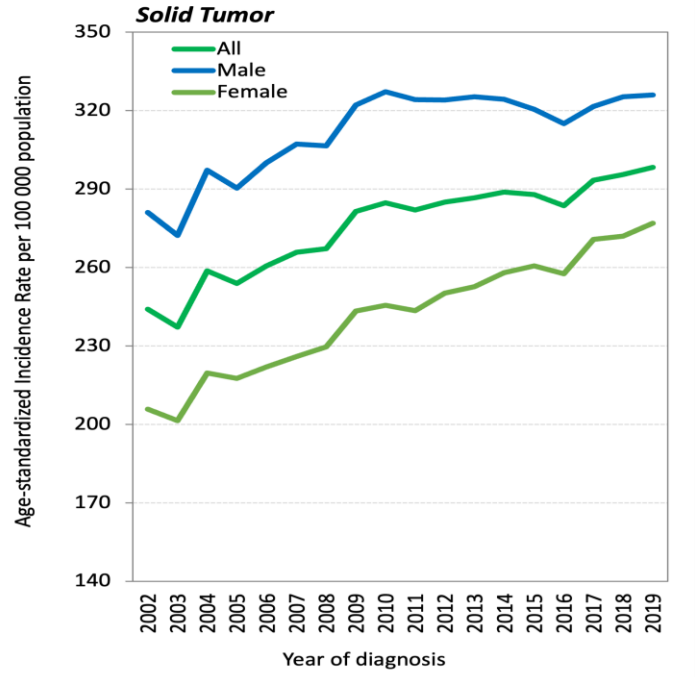
Results: A total of 1,578,049 pts were included (URS [n=2,154], NURS [n=32,454], and NSST [n=1,543,441]). Among all sarcoma pts, 6.2% were URS; the top three most common histologies of URS were endometrial stromal sarcoma (n=292), pleomorphic liposarcoma

(n=212), and epithelioid sarcoma (n=209). The APC of URS continued a surging trend from 2002 to 2019 (APC 3.81, $p < 0.05$) while the APC of NURS and NSST decelerated after 2010. URS pts are younger ($p < 0.001$) and more likely to be female ($p < 0.001$). In the multivariate analysis adjusted for gender, age, and diagnosis period, URS pts had worse OS than NURS pts (HR 1.70, 95% CI 1.59-1.81, $p < 0.001$). When all pts were divided into four age groups (0-18, 18-40, 40-70, and >70), the negative impact of URS on OS was most significant in adolescents and young adults (AYA, age 18-40) (HR:2.23; 95% CI, 1.94-2.55; $p < 0.001$). Unlike NSST, where the OS improved in the past decade (HR:0.84, 95% CI, 0.83-0.84, $p < 0.001$), OS of URS and NURS both worsened (URS: HR:1.14, 95% CI, 1.01-1.30; $p < 0.05$; NURS: HR:1.05, 95% CI, 1.02-1.09, $p < 0.005$) in the period 2011 to 2019 compared to 2002 to 2010.

Conclusion: Our findings showed that the incidence of URS is increasing but the OS worsened. Among different age groups, the detrimental effect of URS was most prominent in the AYA pts. Awareness of URS and precision medicine-based therapies for URS are desperately needed.




Non ultra-rare Sarcoma
All
Male
Female
Age-standardized Incidence Rate per 1 000 000 population
75
70
65
60
55
50
45
40
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
Year of diagnosis


Solid Tumor
All
Male
Female
Age-standardized Incidence Rate per 100 000 population
350
320
290
260
230
200
170
140
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
Year of diagnosis

	Ultra-rare STS (per 1,000,000 population)				Non ultra-rare sarcoma (per 1,000,000 population)				Solid tumor (per 100,000 population)				Solid tumor (per 1,000,000 population)		
year	All	Male	Female	year	All	Male	Female	year	All	Male	Female				
2002	2.6	2.1	3.1	2002	56.6	58.4	54.8	2002	244.0	281.0	205.9	2439.6	2810.0	2058.5	
2003	2.8	2.8	2.7	2003	53.7	51.0	56.6	2003	237.2	272.3	201.4	2371.6	2722.7	2014.2	
2004	2.7	3.3	2.1	2004	61.9	60.3	63.4	2004	258.8	297.2	219.7	2587.5	2972.4	2196.8	
2005	3.9	3.5	4.3	2005	59.0	60.5	57.5	2005	254.0	290.4	217.6	2539.7	2904.1	2176.1	
2006	4.9	4.5	5.3	2006	59.0	59.5	58.6	2006	260.6	300.1	222.0	2606.3	3000.7	2219.6	
2007	3.4	3.2	3.7	2007	60.9	63.2	58.5	2007	265.9	307.2	225.9	2658.8	3072.4	2259.4	
2008	4.3	4.2	4.3	2008	63.1	64.6	61.6	2008	267.2	306.6	229.6	2671.8	3066.0	2296.4	
2009	4.2	4.3	4.1	2009	69.1	73.4	65.0	2009	281.4	322.2	243.3	2814.4	3221.9	2433.3	
2010	4.8	4.6	5.0	2010	68.8	68.5	69.2	2010	284.8	327.2	245.6	2848.0	3272.2	2455.7	
2011	4.4	4.2	4.5	2011	66.9	69.2	64.8	2011	281.9	324.2	243.5	2819.3	3241.7	2435.4	
2012	5.1	4.4	5.8	2012	66.3	70.0	63.1	2012	285.1	324.1	250.3	2850.7	3241.3	2502.6	
2013	5.9	5.0	6.7	2013	62.3	64.6	60.3	2013	286.7	325.3	252.7	2867.0	3253.1	2527.0	
2014	5.2	5.0	5.4	2014	64.7	66.3	63.6	2014	288.8	324.3	258.1	2888.1	3243.0	2580.7	
2015	5.7	5.1	6.3	2015	64.6	65.8	63.9	2015	287.9	320.5	260.6	2879.1	3204.6	2605.9	
2016	6.0	5.7	6.2	2016	61.0	60.3	61.8	2016	283.7	315.0	257.6	2836.6	3149.9	2576.4	
2017	4.8	4.3	5.2	2017	60.7	62.3	59.3	2017	293.3	321.6	270.7	2933.3	3215.6	2707.1	
2018	5.2	4.7	5.6	2018	64.5	67.8	61.8	2018	295.6	325.3	272.0	2956.1	3253.0	2720.3	
2019	5.8	5.5	6.1	2019	65.3	64.8	65.9	2019	298.4	325.9	276.9	2983.8	3259.3	2769.2	

(P 140) **THE PRE-DIAGNOSTIC GENERAL PRACTIONER JOURNEY OF SARCOMA PATIENTS: A REAL-WORLD DATA STUDY.**

Emily Iris Holthuis, MSc[1]; Winette van der Graaf, Prof. Dr.[2]; Cas Drabbe, MD[3]; Winan J. van Houdt, MD, PhD, MS[2]; Yvonne Schrage, MD, PhD[2]; Anne Miek Koenen, n/a[2]; Stephanie van der Kleij, n/a[4]; Marianne Heins, PhD[5]; Olga Husson, PhD[3], (1) Netherlands Cancer Institute - Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (2) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (3) Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands, (4) Netherlands Cancer Institute/Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (5) Nivel, Utrecht, Utrecht, Netherlands

Objective: The majority of cancer patients are diagnosed after experiencing symptoms specific to their cancer type. However, the frequency and the combination of presenting symptoms, also known as the ‘symptom signature’, vary among different cancer types. Due to the atypical presentation of sarcomas and limited experience among healthcare professionals, the diagnostic interval and referral to specialized sarcoma teams can be prolonged and inappropriate treatment might be initiated. Studying the healthcare utilization, e.g. the frequency of consultations and the presence/type of symptoms at the general practitioner (GP), could offer valuable insights into the symptom signature of sarcomas. The NCR-PCD cohort provides a unique platform to investigate the pre-diagnostic trajectory of sarcoma patients within the primary care setting. The available literature on this subject is, to our best knowledge, limited.

Methods: For this study, data from the Netherlands Cancer Registry (NCR) and the Nivel Primary Care Database (PCD) were linked (the NCR-PCD cohort). All adult (≥18 years of age) sarcoma patients diagnosed between 1 January 2010 and 31 December 2020 were identified from the NCR and included in this study. Baseline characteristics were described as mean ±

standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables.

Results: A total of 782 soft tissue sarcoma (STS), 150 bone sarcoma (BS) and 298 gastrointestinal stromal tumor (GIST) patients were identified from the NCR-PCD cohort between 2010 and 2020. The baseline characteristics of the NCR-PCD cohort are presented in Table 1. The mean age at diagnosis (± SD) across the three groups (STS, BS, GIST) was 62.7 ± 16.3 years, 51.1 ± 16.8 years, 66.7 ± 12.9 years, correspondingly. Mean number of GP consultations within the 12-month period preceding diagnosis was as follows: 9.0 ± 7.0 visits for STS patients, 7.6 ± 6.2 consultations for BS patients, 10.5 ± 7.4 consultations for GIST patients. Table 2 displays the top 10 reasons for GP consultation 12-months prior to sarcoma diagnosis. Uncomplicated hypertension, cystitis/other urinary infection and diabetes mellitus are, among others, surprising nonspecific symptoms for which patients consult the GP. All of these symptoms were most frequently seen among STS patients. While other symptoms including neoplasm musculoskeletal and malignant neoplasm stomach are more specific symptoms that align with the respective diagnosis.

Conclusion: Patients with BS and GIST seem to exhibit symptom presentations that align with their respective diagnoses. Conversely, patients with STS seem to present mostly with nonspecific symptoms. Our future research will focus on creating symptom signatures, specific to the various subtypes of sarcoma, observed in the primary care setting. This could, in the future, potentially improve diagnostics and referral practices for these rare cancers.

Table 1. Characteristics of the NCR-PCD cohort.

	NCR-PDC cohort N = 1,230
Gender, male (%)	612 (49.8)
Age at time of diagnosis (years), mean (SD)	62.3 ± 16.2
Histological subtype, no. (%)	
Soft tissue sarcoma	*782 (63.6)*
Dermatofibrosarcoma protuberans	55 (4.5)
Leimyosarcoma	136 (11.1)
Liposarcoma	122 (9.9)
Myxofibrosarcoma	67 (5.4)
Rhabdomyosarcoma	13 (1.1)
Malignant peripheral nerve sheath tumor	9 (0.7)
Vascular sarcoma	58 (4.7)
Synovial sarcoma	22 (1.8)
Kaposi sarcoma	47 (3.8)
Other soft tissue sarcoma	253 (20.6)
GIST	*298 (24.2)*
Bone sarcoma	*150 (12.2)*
Osteosarcoma	34 (2.8)
Chondrosarcoma	102 (8.3)
Chordoma	8 (0.7)
Ewing sarcoma	2 (0.2)
Other bone sarcoma	4 (0.3)
Localization, no. (%)	
Head and neck	25 (2.0)
Thoracic	74 (6.0)
Abdominal excl. urogenital organs	280 (22.7)
Intraperitoneal	239 (19.4)
Retroperitoneal	41 (3.3)
Gynaecological	5 (0.4)
Urological	15 (1.2)
Extremities	350 (28.5)
Upper extremities	98 (8.0)
Lower extremities	252 (20.5)
Breast	56 (4.6)
Pelvis	49 (4.0)
Skin	188 (15.3)
Other localization	183 (14.9)
Unknown	5 (0.4)

Table 2. The top 10 reasons for GP consultation within the 12-month period preceding the diagnosis of sarcoma, with the corresponding frequency (%).

	STS	BS	GIST
1	**Neoplasm musculoskeletal [ICPC: L71]** 158 (32.5)	**Neoplasm musculoskeletal [ICPC: L71]** 68 (45.6)	**Malignant neoplasm stomach [ICPC: 1274]*** 68 (18.9)
2	**Uncomplicated hypertension [ICPC: K86]** 54 (11.1)	**Shoulder symptoms/complaints [ICPC: L08]** 16 (10.7)	**Malignant neoplasm digest other/NOS* [ICPC: D77]** 63 (17.5)
3	**Cystitis/other urinary infection [ICPC: U71]** 53 (10.9)	**Leg/thigh symptoms/complaints [ICPC: L14]** 14 (19.4)	**Uncomplicated hypertension [ICPC: K86]** 48 (13.4)
4	**Malignant neoplasm of the skin [ICPC: S77] *** 44 (9.1)	**Knee symptoms/complaints [ICPC: L15]** 10 (6.7)	**Malignancy neoplasm colon/rectum* [ICPC: D75]** 31 (8.6)
5	**General weakness/tiredness/ill-feeling (excl. psychological) [ICPC: A04]** 33 (6.8)	**Uncomplicated hypertension [ICPC: K86]** 8 (5.4)	**Diabetes mellitus [ICPC: T90]** 31 (8.6)
6	**Leg/thigh symptoms/complaints [ICPC: L14]** 32 (6.6)	**Shoulder syndrome [ICPC: L92]** 7 (4.7)	**Iron deficiency anaemia [ICPC: B80]** 25 (6.9)
7	**Diabetes mellitus [ICPC: T90]** 32 (6.6)	**Localized swelling/papules/lump/mass/skin/ subcutaneous tissue [ICPC: S04]** 7 (4.7)	**Cough [ICPC: R05]** 25 (6.9)
8	**Cough [ICPC: R05]** 28 (5.8)	**Diabetes mellitus [ICPC: T90]** 7 (4.7)	**Other localized abdominal pain [ICPC: D06]** 23 (6.4)
9	**Abdominal mass NOS [ICPC: D24]** 26 (5.3)	**Low back symptoms/complaints without radiation (excl. L86) [ICPC: 1603]** 6 (4.0)	**Cystitis/other urinary infection [ICPC: U71]** 23 (6.4)
10	**Localized swelling/papules/lump/mass/skin/subcutaneous tissue [ICPC: S04]** 26 (5.3)	**Chest symptoms/complaints [ICPC: L04]** 6 (4.0)	**Stomach ache/ stomach pain [ICPC: D02]** 22 (6.1)

*=Suspicion of (…).
ICPC: International Classification of Primary Care, STS: soft-tissue sarcoma, BS: bone sarcoma, GIST: gastrointestinal stromal tumor, PHS: Periarthritis humeroscapularis.

(P 141) TREATMENT VARIABILITY IN SURGICAL MANAGEMENT OF PULMONARY OLIGOMETASTATIC SOFT TISSUE SARCOMA

Valerie L. Armstrong, MD[1]; Zhi Ven Fong, MD MPH DrPH[2]; Elisabeth Lim, MS[2]; Yu-Hui Chang, PhD[3]; Samine Ravanbakhsh, MD[2]; Staci Beamer, MD[2]; Krista Goulding, N/A, MD MPH[2]; Nabil Wasif, MD MPH[2]; Chee-chee Stucky, MD[2], (1) Mayo Clinic, Arizona, Phoenix, AZ, (2) Mayo Clinic Arizona, Phoenix, AZ, (3) Mayo Clinic Arizona, Scottsdale, AZ

Objective: Soft tissue sarcomas (STS) are rare cancers with poor prognosis requiring multidisciplinary management for both localized and metastatic disease. Prior studies have suggested variability in treatment of primary sarcoma, and metastasectomy has been shown to be associated with improved overall survival. Vulnerable populations are associated with poorer access to specialized teams, and in this study, we hypothesize that similar variability exists in the treatment of oligometastatic STS, particularly with pulmonary involvement.

Methods: The National Cancer Database was queried for patients diagnosed with soft tissue sarcoma oligometastastic to the lung between 2004 and 2020. Patient and facility level demographic data, location of primary STS site, grade, management including chemotherapy, immunotherapy, radiation, metastasectomy, as well as patient outcomes and survival were evaluated. All patients had local primary tumor control (surgical or local destruction). Group comparisons were performed by Chi-Squared and Kruskal-Wallis tests and a multivariable logistical regression model was used to assess for adjusted between-group differences in metastasectomy rate and 90-day survival.

Results: Of 689 patients with STS oligometastatic to the lung, 137 (20%) underwent metastasectomy. A majority of patients were privately insured (44%), followed by Medicare

(38%), Medicaid (12%), other government insurance (1.5%), and uninsured (3.8%). Most patients (60%) were treated at an academic program, followed by comprehensive community cancer programs (20%), integrated network cancer programs (17%) and community cancer programs (3%).

No differences in metastasectomy rates were noted in patient sex, age, ethnicity, education level, income level, Charlson-Deyo score, Commission on Cancer (Coc)-facility type, urban versus rural, or great circle distance (Table 1). However, a difference in metastasectomy rate was noted among different races with 20% of Caucasians, and 13% of Black patients undergoing metastasectomy, which was significant after logistical regression on multivariable analysis (odds ratio [OR] 0.57, p=0.03). Predictors of metastasectomy included type of insurance; private 26%, medicaid 17%, medicare 15%, and uninsured 12% undergoing metastasectomy (p=0.003), however after multivariable analysis the difference was not significant. Poorly differentiated tumors were less likely to undergo metastasectomy (OR 0.27; p=0.008 after multivariable analysis). There were no differences in metastasectomy rates based on radiation, chemotherapy, or immunotherapy treatment. Significantly lower 90-day mortality was seen in patients who underwent metastasectomy (OR 0.26, p=0.004) as well as those who underwent systemic chemotherapy with or without metastasectomy (OR 0.26, p< 0.001) even after controlling for demographic factors and comorbidities (Table 2).

Conclusion: Only 20% of patients with STS oligometastatic to the lung underwent surgical resection. Lower metastasectomy rates were seen among Black patients and those with poorly differentiated tumors. Patients that did not undergo metastasectomy had significantly higher 90-day mortality. These findings highlight the need to address variability in treatment and improve access to quality multi-disciplinary care for all patients with pulmonary oligometastatic STS.

Number (%)	Metastasectomy N=137	No Metastasectomy N=552	P-value
Age (years)			0.08[1]
<60	76 (55%)	260 (47%)	
≥60	61 (45%)	292 (53%)	
Sex			0.49[1]
Female	60 (44%)	224 (41%)	
Male	77 (56%)	328 (59%)	
Race			*0.01*[1]
Native Hawaiian / Pacific Islander	1 (1%)	1 (0.2%)	
Asian	11 (8%)	14 (3%)	
White	112 (82%)	437 (79%)	
Black	12 (9%)	84 (16%)	
Other / Unknown	1 (1%)	14 (3%)	
American Indian / Alaskan Native	0	3 (0.5%)	
Ethnicity			0.33[1]
Non-Hispanic	123 (90%)	495 (90%)	
Hispanic	9 (7%)	47 (9%)	
Primary Payor			*0.003*[1]
Private	79 (58%)	226 (41%)	
Medicaid	14 (10%)	68 (12%)	
Medicare	38 (28%)	221 (40%)	
Uninsured	3 (2%)	23 (4%)	
Other Government	0	10 (2%)	
Facility Type			0.31[1]
Community	5 (4%)	12 (2%)	
Academic	73 (53%)	270 (49%)	
Comp. Comm. Cancer	17 (12%)	95 (17%)	
Integrated Net. Can. Ctr.	16 (12%)	80 (14%)	
Charlson-Deyo Score			0.53[1]
0	111 (81%)	423 (77%)	
1	17 (12%)	79 (14%)	
2	6 (4%)	24 (4%)	
≥3	3 (2%)	26 (5%)	
Neoadjuvant radiation	21 (15%)	77 (14%)	0.68[1]
Adjuvant radiation	20 (15%)	112 (20%)	0.13[1]
Neoadjuvant chemotherapy	31 (23%)	94 (17%)	0.13[1]
Adjuvant chemotherapy	66 (48%)	262 (48%)	0.88[1]
90 Day Mortality	6 (4%)	78 (14%)	*0.004*[2]

[1]Kruskal-Wallis; [2]Chi-Square

Metastasectomy	Comparison	Odds Ratio	95% Confidence Interval		P-value
Race	Black vs. White	0.57	0.30	1.09	*0.03*
	Other vs. White	1.49	0.74	2.99	0.70
Insurance	Medicare/govn vs. private	0.49	0.32	0.76	0.13
	Medicaid vs. private	0.64	0.34	1.22	0.91
	Unknown/uninsured vs. private	0.60	0.24	1.53	0.79
Final grade	Poorly vs. moderatev/well diff	0.48	0.27	0.86	*0.008*
90-day mortality	**Comparison**	**Odds Ratio**	**95% Confidence Interval**		**P-value**
Metastasectomy	Yes vs. no	0.27	0.11	0.66	*0.004*
Charlson-Deyo	1 vs. 0	1.0	0.49	2.03	>0.99
	2+ vs. 0	1.48	0.71	3.06	0.29
Any radiation	Yes vs. no	0.63	0.37	1.09	0.10
Any chemotherapy	Yes vs. no	0.26	0.16	0.43	*<0.0001*

(P 142) THE SARCOMA EUROPEAN AND LATIN AMERICAN NETWORK (SELNET) PROJECT: OVERVIEW OF THE MAIN RESULTS OF THE SELNET REGISTRY

Marta Martín-Ruiz, PhD[1]; David S. Moura, PhD[2]; Marta Renshaw, Technician lab[2]; Irene Carrasco Garcia, MD[3]; Juan Carlos Haro Varas, MD[4]; Victor Castro Oliden, Peru[4]; Matías chacón, MD[5]; Andrés Rodriguez, MD[5]; Tomás J. Soule, MD[5]; Claudia Valverde, MD[6]; Celso Abdon Lopes de Mello, MD[7]; Bruna David, MD[8]; Carmen Nieves Hernandez, MD[9]; Josefina Cruz, MD[9]; María Lucila Gónzalez, MD[10]; Laura Cantero-Duerksen, MD[11]; Gema Aguil, MD[12]; Alberto Righi, MD[13]; Marco Gambarotti, MD[14]; Emanuela Palmerini, MD, PhD[15]; Ronald Sergio Limon, MD, MSc[16]; Rodolfo Guzmán-Cervantes, MD[17]; Dorian Yarih García-Ortega, MD, MSc, PhD, FACS[18]; Claudia Haydee Caro, MD[19]; Jorge Luis Martínez, MD[19]; Salvatore Provenzano, MD[20]; Jean-Yves Blay, MD, PhD[21]; Patricio Ledesma, .[22]; Nadia Hindi, MD, MSc[23]; Javier Martin-Broto, MD, PhD[23], (1) Instituto de Investigaciones Sanitarias Fundación Jiménez Díaz, Madrid, Madrid, Spain, (2) Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM),, Madrid, Madrid, Spain, (3) Virgen del Rocio University Hospital, Seville, Andalucia, Spain, (4) Instituto Nacional de Enfermedades Neoplasicas, Lima, Lima, Peru, (5) Instituto Alexander Fleming, Buenos Aires, Buenos Aires, Argentina, (6) Vall d'Hebron University Hospital, Barcelona, Catalonia, Spain, (7) A.C.Camargo Cancer Center., Sao Paulo, Sao Paulo, Brazil, (8) Instituto Nacional de Câncer, Rio de Janeiro, Rio de Janeiro, Brazil, (9) HU Canarias, Santa Cruz de Tenerife, Canarias, Spain, (10) Instituto Nacional del Cáncer, Asunción, Asuncion, Paraguay, (11) Instituto de Previsión Social. Asunción, Paraguay , Fernando de la Mora, Asuncion, Paraguay, (12) Fundación Centro Oncológico de Integración Regional, Mendoza, Mendoza, Argentina, (13) IRCCS, Istituto Ortopedico Rizzoli, Bologna, Emilia-Romagna, Italy, (14) Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (15) IRCCS Istituto

Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (16) OncoBolivia, Santa Cruz de la Sierra, Santa Cruz, Bolivia, (17) Caja Costarricense de Seguro Social, Heredia, Heredia, Costa Rica, (18) Instituto Nacional de Cancerología (México), Tlalpan, Distrito Federal, Mexico, (19) The National Cancer Institute, Cuidad de Mexico, Estado de México, Mexico, (20) Mesenchymal and rare cancer Medical Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (21) Centre Léon-Bérard, Lyon, Rhone-Alpes, France, (22) Sofpromed, Palma de Mallorca, Islas Baleares, Spain, (23) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain

Objective: The Sarcoma European and Latin American NETWork (SELNET) project is an international initiative, funded by the H2020 program, which seeks to create a network of sarcoma expert centers in Latin America (LATAM), with the final aim of improving prognosis of sarcoma patients in this territory. One of the pitfalls in sarcoma management is the lack of reliable epidemiologic data due to the absence of sarcoma registries. Among the several activities of SELNET, a web-based international registry has been started, in order to be used as an institutional database for SELNET centers as well as a sarcoma registry in LATAM. Here we present the preliminary activity of this registry.

Methods: A web-based international registry was designed. Prospective adult patients with soft-tissue sarcoma (STS), bone sarcoma (BS), or GIST, as well as retrospective cases diagnosed since 2005, could be enrolled. European centers could also register patients in the platform. Approvals from ethics committees were obtained in all participating sites. Pseudo-anonymized demographics (gender, date of birth), tumor characteristics (location, size, histologic diagnosis), therapy and outcome data were collected.

Results: From July 2019 to June 2023, a total of 4,469 cases have been registered, with 2,345 (52.4%) patients from LATAM and 2,124 (47.5%) from Europe, belonging to 27 Latin American and 15 European sites. For the entire series, 48.2% were male and 51.7% female, with median age at diagnosis 52 years (18-95). The current global sarcoma type distribution is STS 82.3%, BS 10.2%, and GIST 2.9% (data entry still ongoing).

Conclusion: The SELNET registry represents the first international sarcoma database in LATAM. SELNET's interdisciplinary approach and collaboration among research centers in both LATAM and Europe strengthen the project's impact. The distribution of registered centers between LATAM and Europe demonstrates the project's global reach and enables a diverse representation of patient populations. This project paves the way for similar collaboration models in other rare tumors in LATAM.

(P 143) EXPLORING HOSPITAL VOLUME THRESHOLDS FOR IMPROVED SARCOMA TREATMENT ACROSS DIFFERENT AGE CATEGORIES IN THE UNITED STATES

Charbel Chidiac, MD[1]; Christine A. Pratilas, MD[1]; Adam S. Levin, MD[2]; Nicolas J. Llosas, MD[1]; Alejandro V. Garcia, MD[3]; Mark Slidell, MD, MPH[1]; Daniel S. Rhee, MD, MPH[1], (1) The Johns Hopkins Hospital, Baltimore, MD, (2) Johns Hopkins University School of Medicine, Baltimore, MD, (3) The Johns Hopkins University, Baltimore, MD

Objective: Management of sarcomas is inherently complex due to their diverse nature, necessitating multidisciplinary care, challenging surgeries, a range of systemic therapies,

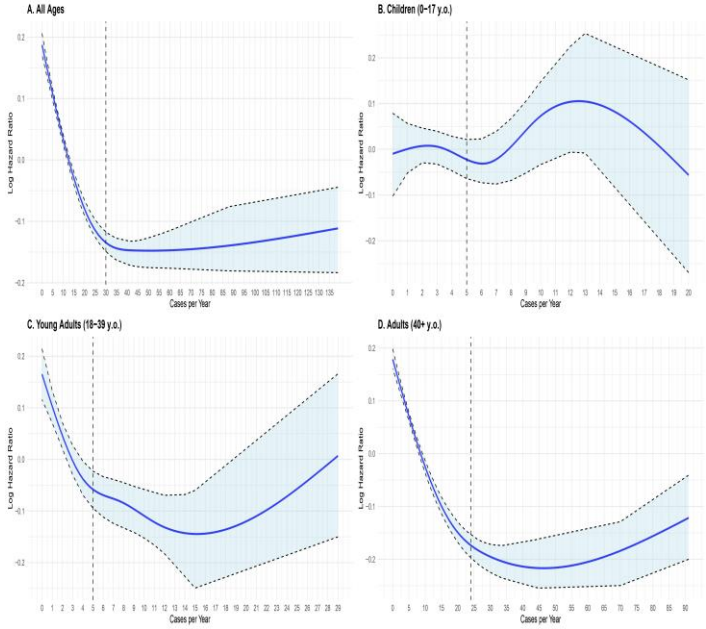
radiation therapy, and long-term monitoring. Prior research has demonstrated that receiving treatment at high-volume hospitals is associated with improved outcomes, including better postoperative results and overall survival rates. Building upon these findings, our study aims to further characterize what hospital volume thresholds in management of sarcoma are necessary for improved outcomes across age groups.

Methods: Patients with Ewing sarcoma, osteosarcoma, rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue sarcoma (NRSTS) who were treated at a single institution were queried from the National Cancer Database (NCDB) from 2004-2019. Patients were further divided into three age groups: children (0-17 years old [y.o.]), young adults (18-39 y.o.) and adults (≥40 y.o.). Multivariate models with restricted cubic splines were used to identify a hospital volume threshold by plotting the number of cases per year against the adjusted hazard ratio. Kaplan-Meyer survival curves and multivariate cox proportional regression accounting for age, sex, cancer type, cancer size and metastasis at diagnosis were then used to test the validity of the extrapolated threshold.

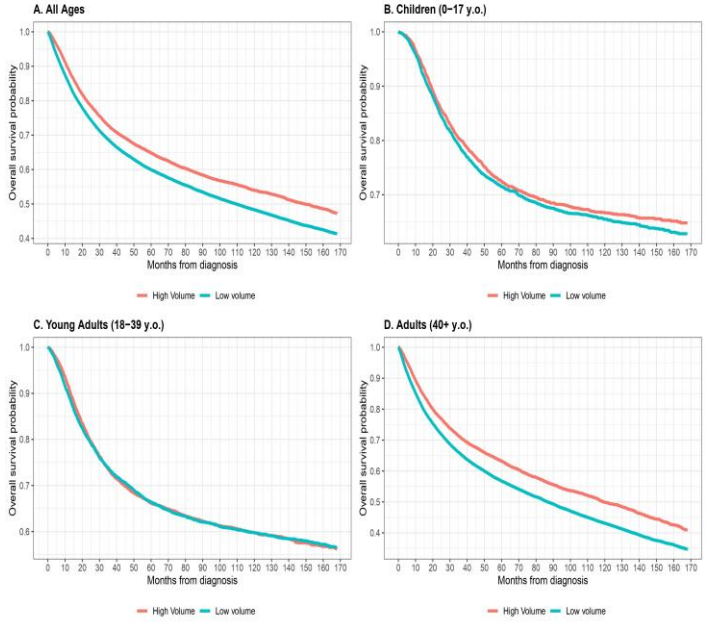
Results: A total of 71,463 patients were included in our study. Among them, 7,829 (10.94%) were children, 12,901 (18.07%) were young adults, and 50,733 (70.99%) were adults. Additionally, 31,569 (44.16%) were female. 4,093 (5.73%) patients had Ewing sarcoma, 55,668 (77.86%) had NRSTS, 8,342 (11.66%) had osteosarcoma, and 3,360 (4.70%) had rhabdomyosarcoma. Median follow up time was 49.5 months. The identified volume thresholds for high-volume hospitals (HVH) were 30 cases per year for patients of all ages, 5 for children and young adults, and 24 for adults. For all ages, 22 out of 1289 hospitals were identified as HVH, treating 25.03% of all patients. For children, 40 out of 443 were HVH, treating 43.73% of the patients. For young adults, 16 out of 970 were HVH, treating 18.18% of patients. For adults, 30 out of 1283 were HVH treating 19.18% of patients. When comparing 5-year overall survival rates, we found that HVH had higher rates of survival compared to non-HVH for all ages (64% vs. 60%), children (72% vs. 71%), and adults (63% vs. 57%). No difference was seen in 5-year OS by hospital volume in young adults (66% vs. 66%) . On multivariate cox proportional regression, being treated at these HVH was associated with increased survival in all groups (for all ages: Adjusted Hazard Ratio (aHR) 0.85, 95% CI 0.83-0.88, p< 0.001; for children: aHR 0.91, 95% CI 0.83-0.98, p=0.032; for young adults: aHR 0.94, 95% CI 0.88-0.99, p=0.048; and for adults: aHR 0.81, 95% CI 0.78-0.85, p< 0.001).

Conclusion: Treatment of sarcoma at HVH is associated with improved outcomes compared to non-HVH in children, young adults, and adults. Our study revealed distinct volume thresholds for hospitals caring for children, young adults, and adults with sarcoma, providing valuable guidance for healthcare professionals for enhancing the referral process and ultimately improving treatment outcomes.. By channeling patients to high-volume hospitals, we can maximize the chances of favorable outcomes.

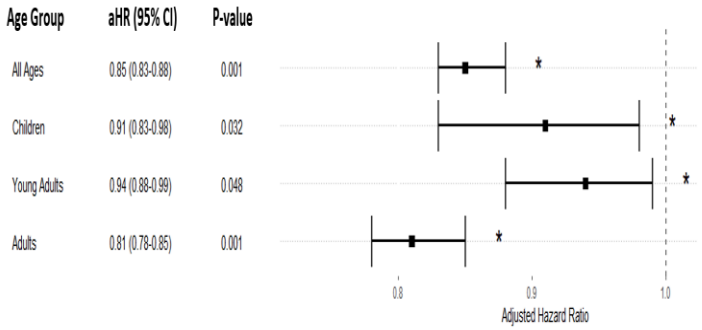
Smoothed Restricted Cubic Spline Plots of the Adjusted Log Hazard Ratio of All Sarcoma Patients
by Average Annual Hospital Volume (Cases per year) by Age Group



Kaplan–Meyer Survival Curves Showing Survival Rates of Sarcoma Patients Undergoing
Treatment at High–volume and Low–volume Facilities by Age Group



Multivariate Cox Proportional Regression Showing the Adjusted Hazard Ratio of Being Treated at a High-Volume Hospital Compared to Being Treated at Low-Volume Hospitals Using The Cutoffs Extrapolated from the Cubic Splines



Variables included in the final model were age, sex, cancer type, cancer size and metastasis at diagnosis.

(P 144) IMPLEMENTATION OF THE EUROPEAN CLINICAL TRIAL REGULATION: SURVEY OF THE ITALIAN SARCOMA GROUP CENTERS

Viviana Appolloni, PhD[1]; Gianluca Ignazzi, Master Degree[2]; Silvia Bastucci[3]; Irene Benzonelli, n/a[4]; Martina Bonel, MSc[5]; Elisabetta Caccin, MSc[6]; Ottavia Clemente, Phd[7]; Daniela Comandini, MD[8]; Elisa Crotti, n/a[9]; Francesca Del Monte, n/a[10]; Simone De Meo, Phd[11]; Caterina Elia, MSc[12]; Nadia Facchinetti, Bachelor[13]; Alfredo Furno, n/a[14]; Giorgia Gentili, n/a[15]; Vincenzo Gennusa, n/a[16]; Stefania Giaquinta, PhD[17]; Teresa Grassani, n/a[18]; Lucia Gravino, MSc[19]; Matilde Ingrosso, MSc[20]; Mariapaola Lisi, MSc[21]; Silvia Lombardi, MSc[22]; Michela Pierini, Phd[23]; Maria Grazia Pionelli, MD[24]; Camilla Rosa, Phd[25]; Daniela Salvatore, Phd[26]; Salvatore Vizzaccaro[27]; Sabrina Zanardi, Phd[28]; Celeste Cagnazzo, Phd[29]; Giacomo Giulio Baldi, MD[30], (1) Italian Sarcom Group, terni, Umbria, Italy, (2) Italian Sarcoma Group, Torino, Piemonte, Italy, (3) IRCCS Istituto Nazionale Tumori Regina Elena, Roma, Lazio, Italy, (4) San Luigi Hospital, Orbassano, Piemonte, Italy, (5) Azienda Ospedale - Università Padova, Padova, Veneto, Italy, (6) Centro di Riferimento Oncologico (CRO), IRCCS, Aviano, Friuli-Venezia Giulia, Italy, (7) Istituto Nazionale Tumori IRCCS Fondazione G. Pascale, Napoli, Campania, Italy, (8) Ospedale Policlinico San Martino-IRCCS, Genova, Liguria, Italy, (9) Istituto Clinico Humanitas Humanitas Cancer Center, Milano, Lombardia, Italy, (10) Nuovo Ospedale Santo Stefano, Prato, Toscana, Italy, (11) Fondazione per la ricerca sui tumori dell'apparato muscoloscheletrico e rari Onlus, Torino, Piemonte, Italy, (12) Centro di Riferimento Oncologico (CRO) IRCCS;, Aviano, Friuli-Venezia Giulia, Italy, (13) Fondazione CNAO - Centro Nazionale di Adroterapia Oncologica, Pavia, Lombardia, Italy, (14) Ospedale Pediatrico Bambino Gesù IRCCS, Roma, Lazio, Italy, (15) IRCCS - Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori" S.r.l., Meldola, Emilia-Romagna, Italy, (16) A.O.U.P. "Paolo Giaccone", Palermo, Sicilia, Italy, (17) IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Emilia-Romagna, Italy, (18) Fondazione Policlinico Universitario Campus

Bio-Medico di Roma, Roma, Lazio, Italy, (19) AOUP Azienda Ospedaliera Universitaria Pisana, Pisa, Toscana, Italy, (20) Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (21) Azienda Ospedaliero Universitaria Careggi, Florence, Toscana, Italy, (22) Istituto Europeo di Oncologia, Milano, Lombardia, Italy, (23) IRCCS Istituto Ortopedico Rizzoli, Bologna, Emilia-Romagna, Italy, (24) AORN Santobono-Pausilipon, napoli, Campania, Italy, (25) Azienda Ospedaliero-Universitaria A.Meyer, Firenze, Toscana, Italy, (26) Fondazione IRCCS Instituto Nazionale dei Tumori, Milano, Lombardia, Italy, (27) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Veneto, Italy, (28) IRCCS ISTITUTO GIANNINA GASLINI, Genova, Liguria, Italy, (29) AOU Città della Salute e della Scienza Presidio Ospedaliero Infantile Regina Margherita, Torino, Piemonte, Italy, (30) Medical Oncology Department, "Santo Stefano" Hospital, Prato, Italy, Prato, Toscana, Italy

Objective: The European Clinical Trials Regulation 536/2014 (CTR) entered into full application on January 31st 2022, by inducing a holistic revolution in European clinical trials (CTs) management. The CTR-related core change was the new Clinical Trials Information System (CTIS). CTIS represents a unique single-entry point for sponsors and authorities, to use for CTs submission and evaluation and includes a searchable database for healthcare professionals and general public. CTIS is an innovative tool that should simplify the submission of CTs application. In Italy, the implementation of CTR required several regulatory steps at national level and substantial procedural modifications at site level. The Italian sarcoma Group (ISG) has recently created a new federated trial center (FCT) organizational model aimed at promoting the establishment of an expert team in the conduction of clinical studies, to share experiences and expertise. Within this model the Study Coordinators of national reference centers for sarcomas belonging to ISG were also educated to the awareness about critical issues, such as CTR implementation, in academic clinical research environment in order to bridge a gap among institutions and sponsors. We herein report the results of a survey that has been circulated among Italian sites belonging to the ISG and aimed at investigating the compliance and the major challenges faced by affiliated sites in adapting to the CTR.

Methods: In April 2023, an anonymous online survey was distributed to the Study Coordinators of the ISG Federated Trial Center and remained active for 2 weeks. All data were anonymous, and participation was voluntary. The questionnaire included 5 macro-areas, with 40 questions overall, assessing institutional awareness and compliance for each item: internal site current organization, management of a new clinical trial submission, assessment of local feasibility, experience with CTIS portal use and future perspectives. Survey was designed with closed-ended questions including binary answers (yes/no), a-single-choice-option list, answers using an ordinal scale (1-10). These latter ones were used to investigate on self-assessments or capabilities of one’s facility and results were statistically analysed with mode and median. A cross-tabulation analysis was used to evaluate responses

Results: 30/48 sites (62,5%) completed the survey. 21/30 were more than sufficiently (conscious median=6.5; mode=7) about the changes introduced by CTR. 50% of responders (n= 15/30) declared that they autonomously found out the training useful for their preparation. 30 responders rated their sites ability to align with the new timeline, to obtain signed study agreement between sponsor and participating site, as not sufficient enough (median=5; mode=5). 25 out of 30 (83,3%) of the sites answered the CTIS related questions. The awareness rate of the new CTR procedures on drafting documentations was complete in 88% of the sites (n=22/25), whereas CTR details for anonymization of personally protected data and commercially confidential information in the clinical documents, requested for a public presentation in CTIS, was known by 72% (n=18/25). Moreover, 72% of responders (n=18/25) confirmed that a dedicated staff, was identified and specifically trained on CTIS use within their

group, while other 16% (n=4/25) were going to plan to do so. On April 2023, 11/30 (36.6%) of participants had already submitted new studies through the CTIS and, in 10 of 11 (90.9%) the submission was accomplished by the in-house staff while in 1/11 was made by an external organization.

Conclusion: Our results showed that most of the research staff belonging to the Italian sarcoma centres have correctly acquired the CTR required skills. Moreover, Italian academic research can rely on its staff, which received high-profile training on CTIS use. This provides the background to the conduction of future academic studies, among which in particular the investigator-initiated ones that are involved in ISG projects. In conclusion an organisational model such the FCT of ISG can be considered as a possible way to support future academic research within the context of the new CTR.

(P 145) OUTCOMES OF BREAST ANGIOSARCOMA UNDERGOING TREATMENT IN A SPECIALIST CENTRE

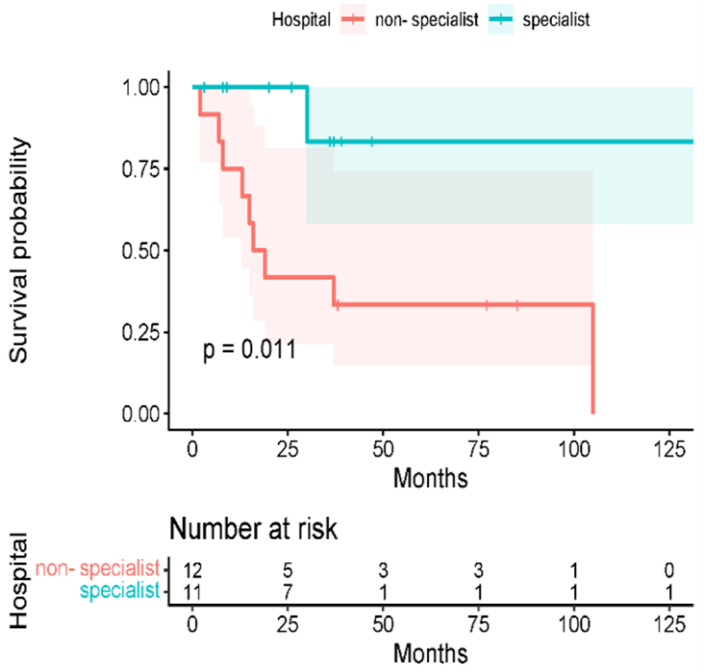
Anjum Shah Mostofa[1]; Mahbubl Ahmed, MRCP PhD FRCR[2], (1) Univerity College London and King's College London, Luton, England, United Kingdom, (2) University College London Hospital, London, England, United Kingdom

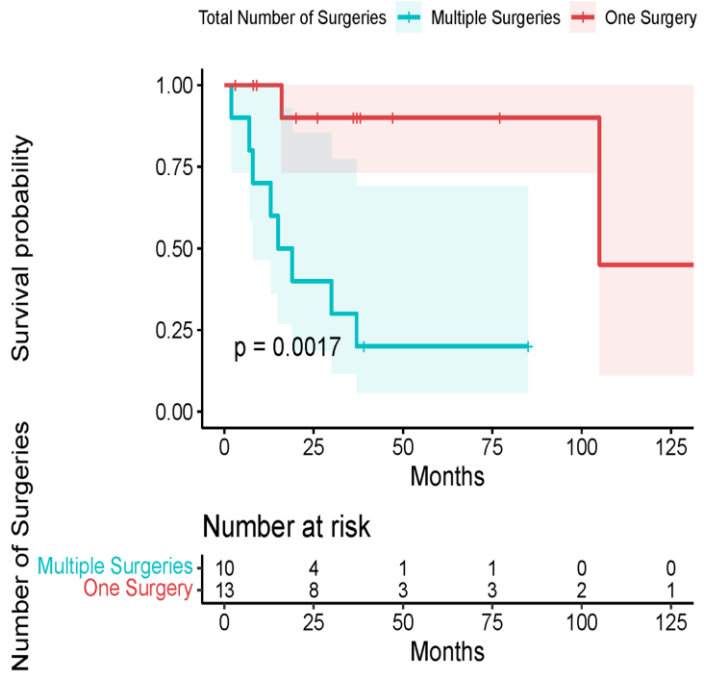
Objective: Breast angiosarcoma (BAS) is a rare cancer and typically occur secondary to radiotherapy of the breast. NHS England service specification has recommended early referral to sarcoma multi-disciplinary meetings. Surgical resection is the most important component of treatment. Clinical outcomes are known to be improved when treatment is undertaken by a specialist multidisciplinary team (MDT). However, there are no studies that compare patient outcomes in specialist centres compared to non-specialist centres. This retrospective study will compare outcomes when treatment takes place in a specialist hospital (containing a sarcoma MDT) to non-specialist hospitals (does not host a specialist's sarcoma MDT).

Methods: All patients diagnosed with BAS and referred to London Sarcoma Services between 2008-2022 were identified from clinical databases. Patient characteristics and clinical outcomes were analysed using Fisher's exact test and Kaplan-Meier curves.

Results: 23 women underwent surgery as their primary treatment (12 non-specialist group v 11 specialist group). In non-specialist hospitals, more patient developed local recurrence (LR) and metastasis (p=0.003 and p=0.027 respectively). Local progression-free survival (L-PFS) was significantly longer in the specialist group v non-specialist group: mean 44.75 months (95% CI 18.73-70.77) versus 115 months (95% CI 15.52–84.58) respectively (P = 0.011) (figure 1). Significantly more patients required >1 surgery in the non-specialist group compared to the specialist group, with longer L-PFS observed in patients receiving only 1 surgery (median 105 (95%CI 0-229.71) versus 15 (95%CI 5.703-24.297) (p= 0.002)) (figure 2). There was no impact on overall survival between the 2 groups.

Conclusion: Undergoing initial BAS surgery in a specialist hospital reduces the risk of LR, metastasis and for multiple operations. Longer L-PFS are observed in specialist hospitals. Due to short follow up there was no difference in survival. Therefore, treatment whenever possible should take place in specialist centres to improve outcomes.


Hospital
non- specialist
specialist
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.011
0
25
50
75
100
125
Months
Number at risk
Hospital
non- specialist
specialist
12
11
5
7
3
1
3
1
1
1
0
1
0
25
50
75
100
125
Months


Total Number of Surgeries
Multiple Surgeries
One Surgery
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.0017
0
25
50
75
100
125
Months
Number at risk
Number of Surgeries
Multiple Surgeries
One Surgery
10
13
4
8
1
3
1
3
0
2
0
1
0
25
50
75
100
125
Months

(P 146) CHARACTERIZING THE PATIENT JOURNEY TO A DIAGNOSIS OF DESMOID TUMOR: DESMOID TUMOR RESEARCH FOUNDATION NATURAL HISTORY STUDY

Shengfan Zhou, MS[1]; Amanda Lucas, BS[2]; Lynne Hernandez, BA[3]; Timothy Bell, MHA[4]; Sandra Goble, MS[1]; Kelly Mercier, PhD[5]; Mitchell Allen, (1) SpringWorks Therapeutics Inc, Stamford, CT, (2) Desmoid Tumor Research Foundation, Jacksonville Beach, FL, (3) Desmoid Tumor Research Foundation, Philadelphia, PA, (4) SpringWorks Therapeutics, Inc., Stamford, CT, (5) Desmoid Tumor Research Foundation, Cary, NC

Objective: Desmoid tumors (DT) are rare, locally invasive, infiltrative, and often aggressive soft-tissue tumors characterized by proliferative growth leading to potentially serious morbidity. They can affect vital structures with high rates of local recurrence, resulting in substantial clinical burden that adversely impacts patients' health-related quality of life. Diagnosing DT is challenging because of its rarity, variable clinical presentation, and heterogeneous morphology. The objective of this analysis from the largest international DT registry and natural history study is to characterize the patient journey to diagnosis.

Methods: The Desmoid Tumor Research Foundation (DTRF) Natural History Study includes surveys for all National Organization Rare Disorders (NORD) participating patient advocacy groups that describe the course of rare diseases from initial symptoms through different clinical stages. Additionally, the DTRF Natural History Study investigators designed several surveys to ascertain information specific to DT and the chronic nature of the disease. This analysis uses data from September 2017 until the cutoff date (October 2022). Eligibility was self-reported DT diagnosis by the study participants and/or their caregivers. This analysis described continuous variables using standard summary statistics and categorical variables using proportions. Chi-square test was used to test proportions between categorical variables.

Results: A total of 332 participants (93% adult, 5% pediatric) completed the DT diagnosis survey: mean age 39 years (median 38; interquartile range [IQR]: 31-48; range: 2-78). Participants were predominantly female (70%), located in North America (88%), and white (86%).

Most (79%) of the participants were diagnosed between 2011-2022. At diagnosis, the most common signs and symptoms were unexplained bumps (59%), pain (57%), fatigue (15%), and change in mobility (13%). Table 1 presents the distribution of the signs and symptoms by DT location. The most common primary methods used to diagnose DT included biopsy (58%) and surgical resection (14%); most participants (92%) had multiple tests (median 4; IQR 3-5; range: 2-9).

Participants experienced high rates of misdiagnosis (n=127, 38%) with broad misdiagnosed conditions; initial misdiagnoses included sarcoma (26 [20%], including gastrointestinal stromal tumor), other cancers (24 [19%], eg, lymphoma, vascular tumor, breast cancer), and various benign tumors or other conditions (83 [65%], eg, muscle injury, lipoma, colon polyps, post-op scars, early onset arthritis, hernia, baker's cyst). Among those who were misdiagnosed, 58% had a tumor ≥5 cm in diameter, 54% had extra-abdominal tumors, and 82% were unifocal at diagnosis. Among the 110 of 127 misdiagnosed participants who completed the surgical history survey, 45% were treated with surgery prior to DT diagnosis. A higher proportion of female participants received a misdiagnosis compared with males (43% vs 28%, P=0.008).

Conclusion: Patients may undergo multiple diagnostic tests and experience burdensome symptoms, such as pain, fatigue, and decreased physical functioning on their journey to a

diagnosis of DT. Approximately 40% of participants reported a prior misdiagnosis, with nearly half receiving surgery prior to DT diagnosis, suggesting that a timely and correct diagnosis is critical to ensure that patients receive appropriate disease management. Raising DT disease awareness addresses a major challenge to achieving an early and accurate diagnosis, thereby facilitating appropriate DT management according to established guidelines.

Table 1: Signs and symptoms at DT diagnosis by tumor location in all participants (N=332)

Signs and symptoms	Tumor location, n (%)				
	Overall* (N=332)	Abdominal wall (n=65)	Intra-abdominal (n=68)	Extra-abdominal* (n=183)	Other location(s) (n=32)
Pain	188 (57%)	39 (60%)	31 (46%)	113 (62%)	15 (47%)
Change in mobility	44 (13%)	4 (6%)	3 (4%)	37 (20%)	4 (13%)
Bleeding	15 (5%)	3 (5%)	9 (13%)	8 (4%)	0
Unexplained bump	195 (59%)	46 (71%)	17 (25%)	122 (67%)	20 (63%)
Headache	21 (6%)	3 (5%)	8 (12%)	15 (8%)	0
Vomiting	34 (10%)	7 (11%)	15 (22%)	14 (8%)	1 (3%)
Fatigue	50 (15%)	14 (22%)	13 (19%)	26 (14%)	2 (6%)
Other	18 (5%)	3 (5%)	8 (12%)	7 (4%)	3 (9%)
No symptoms (tumor found incidentally)	46 (14%)	7 (11%)	19 (28%)	13 (7%)	6 (19%)

*Participants could have multiple symptoms or tumor locations at diagnosis.
Note: Extra-abdominal includes head, neck, extremity, and chest wall. The percentage of symptoms within each tumor location was calculated based on the number of patients with corresponding DT tumor location.

(P 147) UNDERSTANDING THE EXPERIENCES OF PATIENTS RETURNING TO DAILY ACTIVITES AND EXERCISE FOLLOWING LIMB SPARING SURGERY FOR BONE OR SOFT TISSUE SARCOMA AROUND THE KNEE

Emily Dore-Smith, MSc[1]; Sherron Furtado, PhD[2]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[3]; Catherine Pendegrass, n/a[4]; Cecilia Vindrola, n/a[5]; Teja Joshi, MSc[3]; Stephen JG Taylor, MSc PhD[6], (1) University College London, Hampshire, England, United Kingdom, (2) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom, (3) Royal National Orthopaedic Hospital, London, England, United Kingdom, (4) University College London, Greater London, England, United Kingdom, (5) UCL, London, England, United Kingdom, (6) University College London, Stanmore, England, United Kingdom

Objective: The aim of this study is to understand the experiences and potential barriers of returning to daily activities and exercise following treatment for knee sarcoma to pre-operatively guide patient expectations and support rehabilitation.

Methods: In phase one, a narrative literature review was undertaken with sarcoma survivors, with a focus on knee tumours. This informed phase two, a qualitative study of eight patients who were at least one-year post limb sparing surgery of the knee, identified through clinics and clinical records between 2018-2022. These patients had all received their surgery through the

London Sarcoma Service and were purposively sampled. Semi-structured interviews were performed and ethical approval was granted from a National Ethics Committee in the UK (reference 21/WA/0027). Data was then analysed using thematic analysis.

Results: Eight patients with around knee sarcoma were included in the study. Patients received either a distal femoral replacement, proximal tibial replacement or a tibia resection with fibula allograft. Four themes emerged, Navigating activities of daily living, Barriers to exercise, The need for mental health support and Lack of local knowledge. Patients demonstrated resilience returning to life after treatment and expressed both positive and negative aspects of recovery.

Conclusion: Most patients following limb sparing surgery continue to experience not only physical limitations but also long-term effects on mental health. Improved individualised rehabilitation and comprehensive follow up perhaps based on biopsychosocial models, may help to mitigate some of the difficulties faced.

(P 148) MOVING TOWARDS THE OPTIMIZATION OF DIAGNOSIS FOR PATIENTS WITH SARCOMA: A 10-YEAR REVIEW OF EXTERNALLY CONSULTED SARCOMA CASES IN A GENERAL ANATOMICAL PATHOLOGY SERVICE

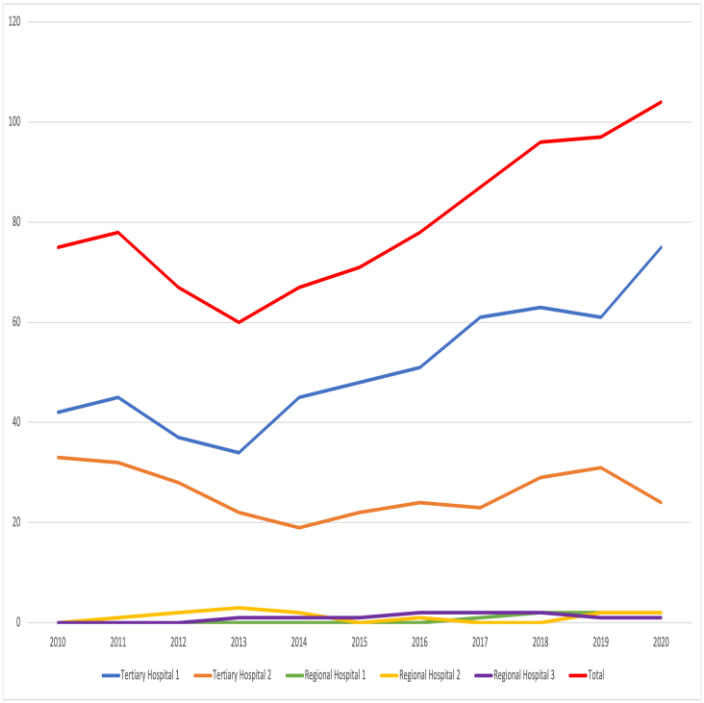
Karan Vats, MD, MSc; Megan Spafford, MD; Gary Groot, MD, PhD, FRCSC, FACS; Peter Graham, MD, FRCSC; Tamalina Banerjee, MD, FRCPC; Ray Deobald, MD, FRCSC; Allison Osmond, MD, MSc, FRCPC, University of Saskatchewan, Saskatoon, SK, Canada

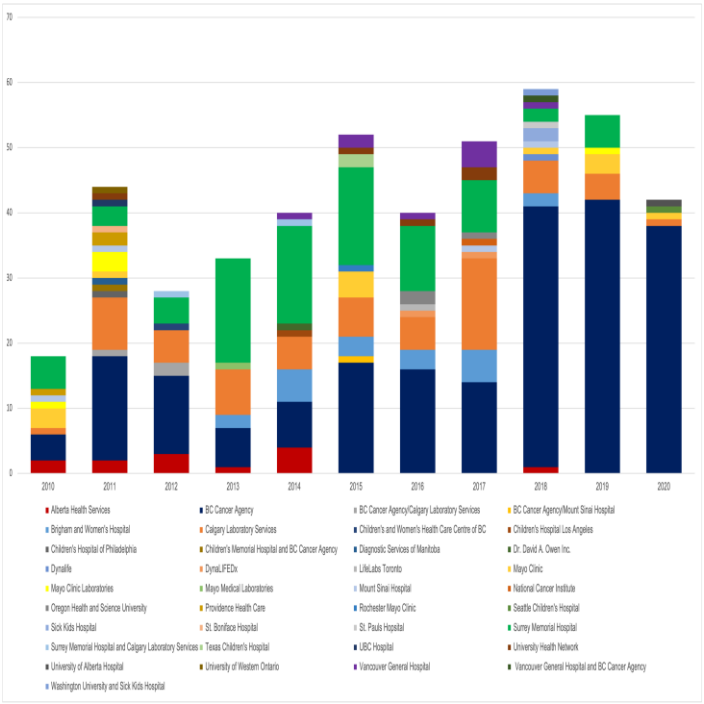
Objective: Soft tissue sarcomas (STS) are rare, diagnostically challenging, malignant tumors with diverse histomorphologic, immunohistochemical and molecular features. In our practice, STS are reported in a general anatomical pathology practice with no formal subspecialized training in reporting these complex specimens. Our study was performed to look at the rate of external consultation (EC), along with other parameters including discordance rate, associated diagnostic delay with EC and extent of secondary work-up performed by the consultant for correct diagnosis.

Methods: The reports from 880 soft tissue sarcomas cases in the province of Saskatchewan between January 1, 2010, to December 31, 2020, were analyzed descriptively.

Results: Of the 880 cases reviewed in our database, 51.9% (n=457) cases were sent to 35 different North American institutions for expert opinion. The initial diagnosis and expert opinion were in full agreement for 182 cases (39.8%), while 194 cases (42.5%) had partial agreement and 66 cases (14.4%) had zero agreement. Of the cases that had zero agreement, 20 cases (4.4%) were initially diagnosed as malignant, with a benign opinion given by the expert; and 10 cases (2.2%) were initially diagnosed as benign, which were malignant upon expert review.

Conclusion: Soft tissue sarcomas are complex tumors that frequently require expert opinion and integration of ancillary techniques with histomorphologic features for definitive classification. A multidisciplinary, subspecialized approach to STS and availability of necessary ancillary tests would improve diagnostic accuracy. In centers where the case load would not support the full-time expertise of an STS multidisciplinary team, criteria should be developed to effectively utilize EC practices.


120
100
80
60
40
20
0
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
Tertiary Hospital 1
Tertiary Hospital 2
Regional Hospital 1
Regional Hospital 2
Regional Hospital 3
Total


70
60
50
40
30
20
10
0
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
Alberta Health Services
BC Cancer Agency
BC Cancer Agency/Calgary Laboratory Services
BC Cancer Agency/Mount Sinai Hospital
Brigham and Women's Hospital
Calgary Laboratory Services
Children's and Women's Health Care Centre of BC
Children's Hospital Los Angeles
Children's Hospital of Philadelphia
Children's Memorial Hospital and BC Cancer Agency
Diagnostic Services of Manitoba
Dr. David A. Owen Inc.
Dynalife
DynaLIFEDx
LifeLabs Toronto
Mayo Clinic
Mayo Clinic Laboratories
Mayo Medical Laboratories
Mount Sinai Hospital
National Cancer Institute
Oregon Health and Science University
Providence Health Care
Rochester Mayo Clinic
Seattle Children's Hospital
Sick Kids Hospital
St. Boniface Hospital
St. Pauls Hospital
Surrey Memorial Hospital
Surrey Memorial Hospital and Calgary Laboratory Services
Texas Children's Hospital
UBC Hospital
University Health Network
University of Alberta Hospital
University of Western Ontario
Vancouver General Hospital
Vancouver General Hospital and BC Cancer Agency
Washington University and Sick Kids Hospital

(P 149) PSYCOLOGICAL SNAPSHOT OF SARCOMA PATIENTS FROM AN ACADEMIC, UNIVERSITY CENTER, HOSPITAL CLÍNICO SAN CARLOS (HCSC), MADRID, A SARCOMA NATIONAL REFERRAL CENTRE IN SPAIN

Gloria Marquina, MD, PhD[1]; Maria Florencia Lira, Psychologist[2]; Juan Antonio Cruzado, Psychology[3]; Celia Ibáñez del Prado, Psychologist[4]; Estefania Dennisse Yangua Paucar, Psychologist[5]; Antonio Casado, MD[6]; Arancha Manzano, MD, PhD[7]; Ana Paula Leiva, Nurse[8]; Florentino Hernando, MD, PhD[1]; Elena Fernández, MD[9]; Juan Luis Cebrian, MD, PhD[10]; Roberto Garcia-Maroto, MD, PhD[8]; Elena Manrique, MD[11]; Javier Buendia, MD[8]; Concepcion Cuenca, MD[8]; Laura Valles, Occupational therapist[8]; Maria Jose Moreno, MD, PhD[8]; Ana Maria Crespo, MD, PhD[8]; Ramiro Mendez, MD, PhD[8]; Marta Garcia, MD[8]; antonio Ruiz, MD[8]; Alejandro Urbina, Md[8]; Luis Ortega, MD[1]; Jose Antonio Cortes, MD[8]; Juan Antonio Corona, MD[8]; Anxela Doval, MD[7]; Pablo Talavera, MD[8]; Esteban Martin-Antona, MD[8]; Maria Cabrera, MD, PhD[8]; Ana Villar Vázquez, Psychologist[12], (1) Hospital Clínico San Carlos, Madrid, Madrid, Spain, (2) Universidad Complutense de Madrid, Santiago, Region Metropolitana, Chile, (3) Universidad Complutense de Madrid, Madrid, Madrid, Spain, (4) Complutense University, Pozuelo de Alarcón, Madrid, Spain, (5) Complutense University, Madrid, Madrid, Spain, (6) Hospital Universitario Clínico San Carlos, Madrid, Madrid, Spain, (7) HU Clínico San Carlos, Madrid, Madrid, Spain, (8) Hospital Clinico san Carlos, Madrid, Madrid, Spain, (9) Hospital Clínico San Carlos, Las Rozas de Madrid, Madrid, Spain, (10) Hospital Clinico San Carlos, Madrid, Madrid, Spain, (11) Hospital Clinico San Carlos, Spain, Madrid, Spain, (12) Hospital Clinico san Carlos, madrid, Madrid, Spain

Objective: Sarcomas are cancers that originate in the bones or soft parts of the body. According to data collected by the Spanish Sarcoma Research Group (GEIS), these diseases do not exceed 1% of all cancers diagnosed in Europe. Like any cancer diagnosis, sarcomas generate emotional impact and distress in patients and their families, affecting their quality of life in multiple dimensions. Hospital Clinico san Carlos is a national referral centre for sarcomas in Spain and our sarcoma MDT has recently incorporated two psychologists within sarcoma clinics to improve sarcoma care. Objectives: To evaluate the levels of anxiety, depression, quality of life, acceptance of the disease and their relationship with sociodemographic and clinical variables in patients with sarcoma.

Methods: The ongoing study has a cross-sectional correlational descriptive design expecting to recruit 124 subjects (started in March 2023). Selection criteria: patients treated at Hospital Clínico San Carlos in Madrid, ≥ 18 years of age, able to read and write in Spanish. Patients with a history of psychopathological disorder were excluded. Independent variables studied were the following: age, sex, marital status, employment and educational situation, subtype of sarcoma, stage and treatments received, evaluated by sociodemographic questionnaire. Symptoms of anxiety and depression were assessed with the Hospital Anxiety and Depression Scale (HAD-A and HADS-D), quality of life with the EORTC QLQ-C30, and the Illness Acceptance Questionnaire. The data was collected online and in person.

Results: 34 patients have accepted to participate from March 2023 so far, 50% of which were men; mean age =51.5 years SD= 14.9, range: 21-81); disease stage I according to TNM 2019:: 31%; II: 31%; III: 25% stage IV: 12.5%. The most relevant data encountered have been so far/ The most relevant findings have been so far : The level of acceptance of the disease was high: 34.5 (SD= 6.87), 95%CI= 32-37. HADS-A= 7.34 (SD= 4.39) 95%CI=5.86-8.93) HADS-D= 5.18 (SD= 4.01), 95%CI=3.72-6, 65). 39% present clinical or subclinical anxiety symptoms and 26%

clinical or subclinical depression symptoms. The results in terms of quality of life are as follows: Global QLQ-C30: 66.14 (SD= 23.94) 95%CI=57.251-74.78); QLQ-C30 Global F. Physics: 77.62 (SD=24.28) 95%CI= 66.87-84.38; QLQ-C30 F. Role: 72.92 (SD=31.32) 95%CI= 61.2-684); QLQ-C30 F. Emotional: 65.62 (SD=27.58)) 95%CI=55.7-75.57; QLQ-C30 F. Cognitive: 76.5625 (SD=28.04) 95%CI=66.45-86.67; QLQ-C30 Social F.: 70.83 (SD=29.63) 95%CI=60.15-81.52. The symptoms with the highest means in the QLQ-C30 have been fatigue: 43.0556 (SD=34.88) 95%CI=30.48-55.63); Pain: 35.42 (SD=35.36) 95%CI=22.67-48.16); Insomnia 40.63 (SD=35.66) 95%CI=22.677-53.48). The patients who received chemotherapy presented lower acceptance (p=0.003), higher levels of depressive symptoms (p=0.001); scored worse in QLQ-C30 in F. Role (p=0.005) emotional (p=0.034) F. Cognitive (p= 0.3021), as well as in fatigue p=0.024) The acceptance variable was highly correlated with HAD-A (r=-0.53) and HADS-D (r=0.55) and showed correlations greater than 0.4 in all dimensions of the QLQC-30.

Conclusion: The intervention has detected high levels of anxiety, depression and quality of life problems in sarcoma patients so far, despite the small size to date. Acceptance scale shows a high reliability, and it is predictive of anxiety, depression and quality of life impairment. Type of treatments influence these parameters, being chemotherapy the treatment with a worse impact on the patient's psychological sphere. These preliminary data highlight the need for comprehensive care for patients with sarcoma mainly psychological assessment and intervention.

(P 150) **VISUALISING SOFT TISSUE SARCOMA INCIDENCE DATA FOR PATIENT EMPOWERMENT**

Sarah Kingsmill, .; Sorrel R B Bickley, PhD; Kate E. Quillin, MSc; Enda Guinan, MA, Sarcoma UK, London, England, United Kingdom

Objective: Historically, limited data on the incidence and survival of soft tissue sarcomas in the UK has left patients with uncertainties and a lack of information about their specific subtype within the broader sarcoma landscape.

Recent work by Bacon et al. (2023) analysed soft tissue sarcoma data in England between 2013-2017. This work made available the incidence and survival rates of many soft tissue sarcoma subtypes for the first time and uncovered valuable insights for sarcoma service provision.

We aimed to make these data more widely accessible in an easily understandable and visually engaging format for people affected by sarcoma without a scientific background, and for healthcare professionals.

Methods: We compiled the study’s findings and converted them into a number of visual resources on Sarcoma UK’s website and social media.

An interactive dashboard on Sarcoma UK’s website allowed users to explore and interact with the data. An accompanying website hub provided comprehensive information on incidence, survival, routes to diagnosis and deprivation data for more than 80 subtypes of soft tissue sarcoma.

We also produced several shareable infographics tailored for social media platforms. This includes an ongoing weekly data series which features data for individual subtypes in further detail.

Following the publication of the dashboard, we collaborated with NHS England on a webinar and data story to disseminate and explain the impact of the data to a wider professional audience.

Results: Feedback from the patient, caregiver and healthcare communities on this initiative has been hugely positive. The visual resources garnered a significant number of views and achieved broad reach, across a variety of audiences. The interactive dashboard has emerged as the 6th most viewed webpage on Sarcoma UK's website since its launch in May 2023.

Our infographics shared on social media have also achieved significant reach and engagement, with around 40,000 impressions as of 20th June 2023. The posts sparked conversations among patients, caregivers, and healthcare professionals, fostering a sense of community and knowledge exchange.

Conclusion: The active engagement and interaction among individuals demonstrate the value of presenting sarcoma data in a visually appealing and easily digestible format, for patients, healthcare professionals, and Sarcoma UK as a charity.

(P 151) DEVELOPMENT AND IMPACT OF A PRIMARY CARE SARCOMA EDUCATION MODULE FOR ENGLAND AND WALES

Sorrel R B Bickley, PhD[1]; Bradley J. Price, MSc[2], (1) Sarcoma UK, London, England, United Kingdom, (2) Action for Pulmonary Fibrosis, London, England, United Kingdom

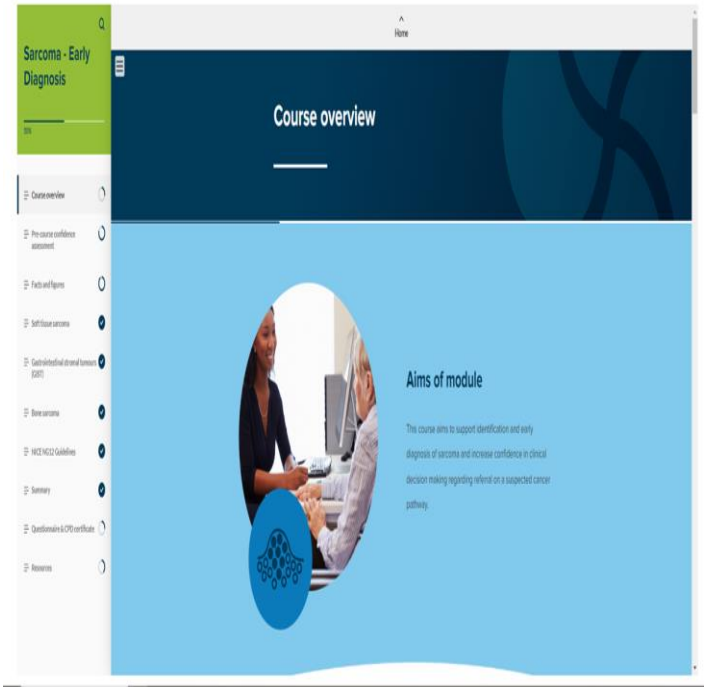
Objective: Patients often report that the biggest delay to their diagnosis is repeated visits to primary care physicians, who doesn't recognise their symptoms of being a potential sarcoma. Data from the UK National Sarcoma Survey in 2020 revealed that a third of all sarcoma patients see a GP more than 3 times before they are referred for further tests, and 1 in 3 patients waited at least six months after first consulting a healthcare professional before receiving an accurate diagnosis. The later sarcoma is diagnosed, the fewer options available for treatment and the higher the chance of that treatment not working. In our policy report, Delays Cost Lives, Sarcoma UK called for an education programme for medical professionals to help improve these statistics.

Methods: Sarcoma UK worked with GatewayC, a free online cancer education platform, to develop a module to educate GPs in England and Wales on the signs and symptoms of sarcoma in the hope to improve early diagnosis, increase sarcoma survival, and enhance patient experience. This involved collaborative development of content through engagement with people affected by sarcoma, sarcoma specialists and primary care physicians. The development of course content included reviews of National guidelines and statistics on sarcoma, summarising details on referral pathways and developing patient case studies to help highlight typical presentations of sarcoma.

Results: The course was developed and is now hosted by GatewayC and aims to support GPs

and primary care professionals in recognising the symptoms of sarcoma, as well as to improve confidence in decision-making regarding referrals on a suspected cancer pathway. The course features interviews with sarcoma specialists and patients themselves. To the end of June 2023, 297 primary care physicians have completed the module, and at launch it was the third most popular course on the platform.

Conclusion: Improving education on sarcoma for primary care physicians is consistently highlighted as a priority for both sarcoma patients and specialist clinicians. The launch of a new sarcoma education module in England and Wales enabled 297 primary care professionals to gain knowledge of sarcoma symptoms and referral pathways in 2022, and it is hoped that other Nations within the UK and Internationally could follow this model.




Sarcoma - Early Diagnosis
55%
Course overview
Pre-course confidence assessment
Facts and figures
Soft tissue sarcoma
Gastrointestinal stromal tumours (GIST)
Bone sarcoma
NICE NG12 Guidelines
Summary
Questionnaire & CPD certificate
Resources
Patient case study
James is a 20 year old male who enjoys playing football. He started experiencing some lower leg pain and swelling. After 18 months, James was diagnosed with osteosarcoma. James' story is based on the experience of a real patient who supported the development of this module. Click through the timeline below to read about his journey.
February 2018
August 2018
December 2018
March 2019


Sarcoma - Early Diagnosis
55%
Course overview
Pre-course confidence assessment
Facts and figures
Soft tissue sarcoma
Gastrointestinal stromal tumours (GIST)
Bone sarcoma
NICE NG12 Guidelines
Summary
Questionnaire & CPD certificate
Resources
AGE
SURVIVAL
TYPES OF SARCOMA
Sarcoma survival rates have been gradually increasing over the last two decades in the UK. 78% of people diagnosed with sarcoma in the UK will live up to a year, with 55% of sarcoma patients living for five years or more.
Sarcoma statistics
Click the icons below
Soft Tissue Sarcoma
Bone Sarcoma

(P 152) EVALUATING DIAGNOSTIC APPROACHES TO DERMATOFIBROSARCOMA PROTUBERANS: AN OPPORTUNITY TO REDUCE THE NUMBER OF INTERVENTIONS

Leah K. Winer, MD[1]; Rita Akumuo, MD[2]; Mengying Deng, MMath[3]; Elizabeth Handorf, PhD[3]; Stephanie H. Greco, MD[4]; Margaret von Mehren, MD[1]; Alex J. Bartholomew, MD, MS[5]; Dan Blazer, III, MD[5]; Lucie Gabrielova, MD[6]; Dagmar Adámková Krákorová, MD, PhD[7]; Natalie Mezey, BSc[8]; Ericka Bernard-Bedard, BSc[8]; Carolyn Nessim, MD[8]; Attila Kollar, MD[9]; Radu Olariu, MD, PhD[10]; Romi Cencelj-Arnez, MD[11]; Daphne Hompes, MD, PhD[12]; Samuel Ford, MD, PhD[13]; Kenneth Cardona, MD[14]; Kenji Sato, MD[15]; Shintaro Iwata, MD[15]; Jeffrey M. Farma, MD[1]; Anthony M. Villano, MD[1], (1) Fox Chase Cancer Center, Philadelphia, PA, (2) Department of General Surgery, Temple University Hospital,, Philadelphia, PA, (3) Division of Biostatistics and Bioinformatics, Fox Chase Cancer Center, Philadelphia, PA, (4) Fox Chase Cancer Center, newtown, PA, (5) Duke University, Durham, NC, (6) Masaryk Memorial Cancer Institute, Brno, Olomoucky kraj, Czech Republic, (7) Masaryk Memorial Cancer Institute Brno, Czech Republic, Brno, Jihomoravsky kraj, Czech Republic, (8) Department of Surgery, University of Ottawa, Ottawa, ON, Canada, (9) Department of Medical Oncology, University Hospital Bern, Inselspital, Switzerland, Bern, Bern, Switzerland, (10) Department of Plastic and Hand Surgery, Inselspital University Hospital Bern, Freiburgstrasse, Bern, Switzerland, (11) Institute of Oncology Ljubljana, Ljubljana, Ljubljana, Slovenia, (12) University Hospitals Gasthuisberg Leuven, Leuven, Vlaams-Brabant, Belgium, (13) Department of Surgery, University Hospitals Birmingham, Birmingham, England, United Kingdom, (14) Department of Surgery, Winship Cancer Institute, Emory University, Atlanta, GA, (15) National Cancer Center Hospital, Tokyo, Tokyo, Japan

Objective: Dermatofibrosarcoma protuberans (DFSP) is a rare soft tissue sarcoma that is difficult to diagnose due to its occult clinical presentation. The National Comprehensive Cancer Network Clinical Practice Guidelines recommend incisional, punch, or core needle biopsy, but there are limited data regarding the optimal diagnostic approach. The objectives of this study were to characterize current practices of DFSP diagnosis and to compare clinical outcomes based on chosen biopsy technique.

Methods: Data were retrospectively collected for adult patients who underwent surgical resection for the initial presentation of a DFSP lesion between 2003 and 2021 at 11 institutions in eight countries. Patients were grouped according to biopsy type: excisional biopsy versus preoperative biopsy, which included incisional, punch, and core needle biopsies. Demographics, tumor characteristics, treatment strategies, and outcomes were explored with descriptive statistics and univariable analyses.

Results: The whole cohort included 366 patients, 86.5% (N=315) of whom were treated at academic centers. The median age was 42 (IQR 31-54) years, and 53.6% (N=195) were male. A DFSP diagnosis was made in 91.6% (N=318) of patients based on biopsy procedure. Among these, 50.4% (N=172) were excisional, 25.5% (N=87) were incisional, 16.1% (N=55) were punch, and 7.0% (N=24) were core needle biopsies. Notably, DFSP was an incidental pathologic finding in 21.7% of all cases (N=78), and preoperative imaging was used to characterize tumors 56.1% of the time. Patients were then compared according to biopsy type (excisional biopsy, N=172; preoperative biopsy, N=166). The proportion who underwent excisional biopsy was relatively stable during the study period according to a time-trend analysis (p=0.057). There were no differences between groups in terms of patient sex, disease presentation, or disease location. Excisional biopsy was most frequently performed by general surgeons (49.1%), as compared with dermatologists (21.0%), surgical oncologists (4.8%), or

primary care physicians (1.2%). Compared with excisional biopsy patients, patients who underwent preoperative biopsies were likely to be younger (median 43 (IQR 36-55) vs 39.5 (IQR 29-53) years, p=0.031) and treated at academic institutions (80.1% vs 91.1%, p=0.009) with their biopsies performed by dermatologists (21.0% vs 44.0%, p< 0.001). Regardless of biopsy type, groups were similar as far as planned circumferential margins at first resection, final pathology, and specimen size. Although excisional biopsy patients required a greater median number of excisions to clear disease (2 vs 1) and were less likely to achieve R0 margin status after the index resection (59.2% vs 77.2%, p< 0.001), final R0 rates were comparable in both groups (93% excisional biopsy versus 87.5% preoperative biopsy, p=0.10). Recurrence was altogether rare but was more frequently observed after excisional biopsy than preoperative biopsy (8.1% vs 4.1%, p>0.05).

Conclusion: In this international, multicenter collaborative, diagnostic approaches to DFSP were quite varied. Excisional biopsy appears to be a reliable diagnostic tool, however, its association with positive surgical margins and multiple excisions may limit its benefit and introduce potential morbidity. Efforts should be made to unify diagnostic approaches to mitigate extraneous procedures and improve outcomes.

(P 153) PRIORITY SETTING PARNTERSHIP RETROPERITONEAL SOFT TISSUE SARCOMAS

Markus Albertsmeier, MD[1]; Markus Wartenberg[2]; Martin K. Angele, MD[1]; Jens Werner, MD[1]; Lars H. Lindner, MD[3]; Nina-Sophie Schmidt-Hegemann, MD[4]; Wolfgang G. Kunz, MD[5]; André Mihaljevic, MD[6]; Jens Jakob, MD[7], (1) LMU University Hospital, Munich, Bayern, Germany, (2) German Sarcoma Foundation (DSS), SPAGN, Woelfersheim, Hessen, Germany, (3) Department of Medicine III, LMU University Hospital, LMU Munich, Munich, Bayern, Germany, (4) Department of Radiation Oncology, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (5) Department of Radiology, University Hospital, Ludwig-Maximilians-University Munich, 81377 Munich, Germany, Munich, Bayern, Germany, (6) Ulm University Hospital, Ulm, Baden-Wurttemberg, Germany, (7) University Medical Center Mannheim, University of Heidelberg, Mannheim, Baden-Wurttemberg, Germany

Objective: Retroperitoneal soft tissue sarcomas present unique challenges due to their anatomical location, late diagnosis, complex management, and limited understanding of their biology. The evidence guiding the diagnosis, treatment, and long-term outcomes of retroperitoneal sarcomas is scarce compared to more common malignancies. The scarcity of evidence stems from the rarity of the disease, the heterogeneity of its presentation, and the lack of dedicated research efforts. Consequently, there is an urgent need to systematically identify and prioritise research questions to guide future investigations and improve the understanding, management, and outcomes of patients with retroperitoneal soft tissue sarcomas. The objectives of this priority-setting partnership are to identify the top 10 unanswered research questions in the study of retroperitoneal soft tissue sarcomas by engaging stakeholders and reviewing the existing literature and to generate hypotheses that can be addressed in clinical trials.

Methods: A collaboration has been established between the sarcoma center at LMU University Hospital Munich, Germany, the research group "Priority Setting Partnerships“ (Ulm University Hospital, Germany), and the German Sarcoma Foundation (a patient advocacy group). The project encompasses a two-year work program consisting of eight work packages (WP),

following recommendations by the James Lind Alliance. WP 1 focuses on Stakeholder Integration, involving identification and contact establishment with various stakeholders. A steering committee comprising representatives from each stakeholder group will oversee the PSP, and an initial meeting of all stakeholders will initiate the partnership officially. WP 2 entails conducting an Initial (Screening) Survey. Surveys will be administered online and in paper format, targeting the public and involved parties. Focus groups with relevant stakeholders will also be organized, and the transcripts will undergo framework analysis. The Initial (Screening) Survey findings will be refined in WP 3 to create a shortlist of prioritized research questions. Recurring topics from the survey will be synthesized into questions within each topic. The shortlisted questions will be further refined through consultation with experts and the steering committee. Work Package 4 involves a Systematic Literature Search to determine if any of the questions have already been addressed, allowing for the exclusion of redundant inquiries. An interim prioritization will be conducted in Work Package 5. This will involve administering a second round of surveys to stakeholders to determine the relative importance and urgency of the questions. Work Package 6 focuses on the Final Prioritization. The project will plan a consensus meeting involving all stakeholders to finalize the prioritization of the research questions. Through structured discussions and facilitated consensus-building processes, agreement will be reached on the most critical research questions. Work Package 7 pertains to the Dissemination of Results. The outcomes of the project will be shared among all stakeholders and partners. Furthermore, the results will be published in a peer-reviewed journal, and presentations will be made at relevant national and international conferences. Public relations efforts will be undertaken through established websites and press releases. Finally, Work Package 8 will be dedicated to Trial Design. The project aims to prepare outlines for three clinical trials that address the prioritised research questions. These trial designs will be developed for submission to a public funding body.

Results: A systematic literature search has revealed that no retroperitoneal soft tissue sarcoma-specific priority-setting partnership has been initiated yet. The steering committee has been formed, and government funding for this project has been secured for a two-year period.

Conclusion: The Priority Setting Partnership Retroperitoneal Soft Tissue Sarcomas has been initiated. It will identify the top 10 research questions in this specific field and translate them into hypotheses that can be tested in clinical trials. National funding has been obtained, and the project is open to include stakeholders from the global sarcoma community.

(P 154) BARRIERS TO CARE FOR MUSCULOSKELETAL TUMORS

Alina Syros, MPH[1]; Jenna Adalbert, MD[2]; Hallie B. Remer, BS[1]; Max Baron, BS[2]; Marilyn Heng, MD MPH[2]; Brooke Crawford, MD[2]; Emily Jonczak, MD[3], (1) University of Miami Miller School of Medicine, Miami, FL, (2) University of Miami, Miami, FL, (3) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL

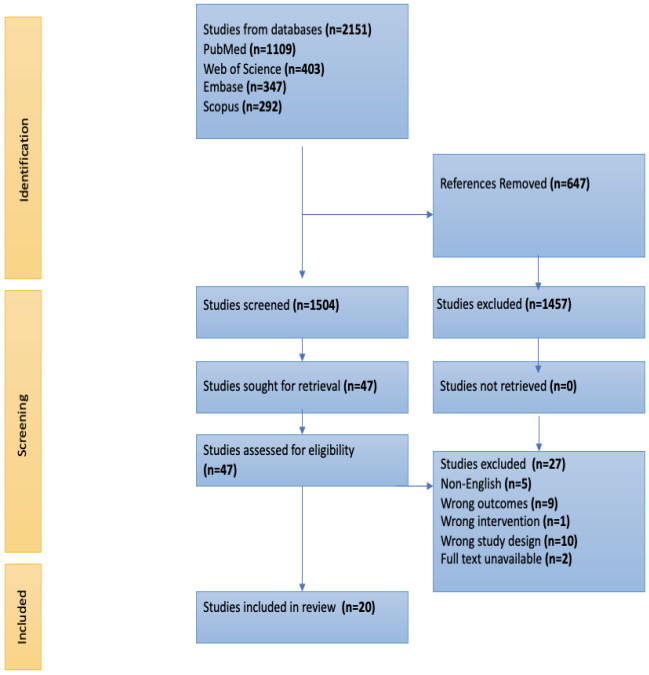
Objective: Disproportionate access to resources and inequal distribution of care creates important delays in the delivery of healthcare. Understanding the delays in care for patients with musculoskeletal tumors is particularly important given the necessity of prompt diagnosis and treatment for oncologic diseases. If barriers to care exist that prevent patients from receiving timely care, malignant disease may progress beyond the limits of treatment. Therefore, we aimed to perform a high-quality systematic review of the literature to determine the most

significant barriers to care that exist for patients undergoing diagnosis and treatment of musculoskeletal tumors.

Methods: A systematic review of the literature was conducted in Scopus, Embase, Web of Science, and PubMed-MEDLINE using a combination of terms. Articles that were in English, available as full text, included patients with musculoskeletal tumors and reported barriers to care experienced by patients were eligible. From these articles, the following data were extracted: musculoskeletal tumor diagnosis, type of barrier to care, patient sex, age, treatment course, and clinical outcomes if available. Quality assessments of final studies were also conducted using the Joanna Briggs Institute's critical appraisal tools.

Results: Searches generated a total of 1504 publications, and 20 articles were eligible for the final analysis. Across the 114,056 patients in the 20 articles, 5 articles (25%) reported on geographic or locational barriers including distance to academic centers, 5 articles (25%) reported on barriers to diagnosis including rural and suburban settings, 5 articles (25%) reported on financial (socioeconomic/insurance) barriers, and 5 articles (25%) reported on barriers related to patient demographics such as marital status, race, and psychologic factors.

Conclusion: Given the community-related barriers that exist for patients with musculoskeletal tumors, key initiatives such as increasing access to referral centers/academic centers within specific patient communities may reduce delays to care for oncologic patients. These pre-hospital barriers to care highlight the importance of public health initiatives focused on improving patient access based on both internal and external patient factors. Further studies are warranted to explore specific interventions that improve patient access and prevent musculoskeletal tumor progression to an untreatable or complex surgical stage.



(P 155) THE IMPACT OF THE ROLE OF A SARCOMA ADVANCED NURSE PRACTITIONER (ANP) BIOPSY CLINIC IN IMPROVING PATIENT ACCESS TO SARCOMA SERVICES

Hazel L. Murray[1]; Mark Doherty, MB BCh BAO MSc MRCPI[2]; Una Hayden, RN HDip MSc[3]; Paul Ridgway, n/a[3]; Amy Gillis, MD MCh[3]; Gary O'Toole, n/a[4]; alan Molloy, n/a[4]; Andrea Marnell, n/a[5]; Aine Sheridan, RGN, PG DIp[4]; Charles Gillham, MRCP FRCR MD[6]; Naomi Lavan, n/a[6]; Eric heffernan, n/a[4]; David Healy, n/a[4]; Aurelie Fabre, n/a[4]; Tom Crotty, n/a[4], (1) St Vincents University Hospital, Elm Park, Dublin 4, Ireland, Dublin, Dublin, Ireland, (2) St Vincents University Hospital, Dublin 4, Dublin, Ireland, (3) Tallaght University Hospital, Dublin, Dublin, Ireland, (4) St Vincents University Hospital, Dublin, Dublin, Ireland, (5) Cappagh National Orthopaedic Hospital, Dublin, Dublin, Ireland, (6) St Luke's Radiation Oncology Network, Dublin, Dublin, Ireland

Objective: Access to soft tissue sarcoma services for the circa 200 new patients per year in Ireland, has improved significantly since 2013 following formalising and centralising Multidisciplinary Team networks. Oncological Survival rates are comparable to near EU/ UK healthcare regions, but timely access to biopsy and diagnostics remains challenging owing to the geographically spread relatively small population. Recently, an NCCP-sponsored Sarcoma Advanced Nurse Practitioner (ANP) led diagnostic clinic (SDC) was set up, in parallel to usual referral pathway. This study was to determine whether the SDC provided more efficient access to biopsy and diagnostic outcomes for patients with suspected/confirmed sarcomas in Ireland.

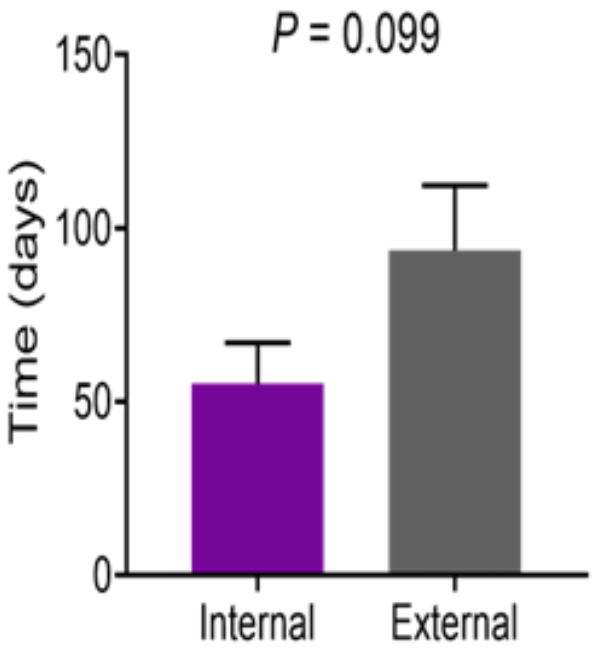
Methods: Sample patient data referred to one of the two SDCs (SVUH) the sarcoma ANP SVUH from Jan 2022 to May 2023 were retrospectively reviewed. Patients who had high clinical/radiological suspicion of sarcoma were offered an ANP examination and handheld 14/16G core biopsy appointment. Upon clinical review to determine suitability for non-image guided cores, the biopsy was performed by the trained ANP supervised by Consultant Surgeon/fellow. Method undertaken was as per standard ESMO guidelines, with the biopsy tract situated within further excisional scar. Intervention outcomes, Patient healthcare records (HCR's), administration systems for time stamps and telephone/email logs were examined.

Results: A total of 35 patients between January 2022 and March 2023 were referred directly to the SVUH SDC and a further 5 were MDT referrals for treatment abroad scheme (TAS) and thus excluded from outcome but not time to MDT analysis. Within 72 hours of referral, 40% (n=14) were contacted by the ANP virtually for clinical history and consideration of imaging. Patients who had high clinical/radiological suspicion of sarcoma were offered a SDC within 2 weeks (100%). Upon clinical review 22% (n=8) had a biopsy performed by the ANP. There were no adverse events apart from expected minor ecchymosis and discomfort noted in this group. The SDC pathways had an average time to MDT of 7 days (range 5-13 days). A sample of 20 patients referred to TUH between 2015 and 2018 were divided equally and analysed with primary end point being time from referral receipt at sarcoma centre to treatment decision in either group. Results demonstrated patients referred from local hospitals with antecedent investigations were associated with almost double waiting time for definitive diagnosis at MDT than those referred from GP etc (See figure 1)

The 5 patients for review at the TAS MDT 100% (n=5) had the relevant proforma sent by the sarcoma ANP within 24 hours after MDM recommendation and an average time to MDT of 14 days (range 7-21 days). Prior to this, referrals were Consultant to Consultant and UK MDM co-ordinators were responsible for obtaining Irish datasets, a process which could take up to 6 weeks.

Conclusion: Soft tissue sarcomata can be selected safely and efficiently for handheld core biopsy in an ANP led sarcoma clinic. This allows quicker access for cases to MDT than standard pathways of care.

Figure 1. Results of TUH patient analysis



(P 156) PATIENT REPORTED EXPERIENCES WITHIN THE IRISH SARCOMA GROUP

Hazel L. Murray[1]; Mark Doherty, MB BCh BAO MSc MRCPI[2]; Una Hayden, RN HDip MSc[3]; Paul Ridgway, n/a[3]; Amy Gillis, MD MCh[3]; Aine Sheridan, RGN, PG DIp[4]; Gary O'Toole, n/a[4]; alan Molloy, n/a[4]; David Healy, n/a[4]; Andrea Marnell, n/a[5]; Eric heffernan, n/a[4]; Aurelie Fabre, n/a[4]; Tom Crotty, n/a[4]; Charles Gillham, MRCP FRCR MD[6]; Naomi Lavan, n/a[6], (1) St Vincents University Hospital, Elm Park, Dublin 4, Ireland, Dublin, Dublin, Ireland, (2) St Vincents University Hospital, Dublin 4, Dublin, Ireland, (3) Tallaght University Hospital, Dublin, Dublin, Ireland, (4) St Vincents University Hospital, Dublin, Dublin, Ireland, (5) Cappagh National Orthopaedic Hospital, Dublin, Dublin, Ireland, (6) St Luke's Radiation Oncology Network, Dublin, Dublin, Ireland

Objective: The Irish Sarcoma Group (ISG) wish to gain insight into patients' experiences particularly regarding areas of awareness, treatment toxicity and survivorship considering the unique and complex needs of this population.

Methods: Between January and March 2023 20 patients previously/currently receiving chemotherapy or on active surveillance were randomly selected to answer a telephone survey developed by ISG members.

Results: 10 males and 10 females were interviewed by the sarcoma advanced nurse practitioner (ANP), with age range 17-47. 90% of patients (n=18) were unaware of the term “sarcoma” prior to diagnosis. 75% (n=15) were given their diagnosis outside the ISG and the term “cancer” was used instead of “sarcoma”. The GP was the first point of contact in 60% (n=12) and physio in 25% but referrals to diagnostics varied from weeks to years in some cases. 25% (n=5) were given their diagnosis by a member of the ISG (surgeon/oncologist/sarcoma ANP) and fully understood the implications and treatment goals. 70% were aware that their case would/was discussed at the National MDM. Nausea, fatigue and alopecia were cited the most distressing treatment toxicities in all patients. Time away from home/loss of independence were the most distressing treatment impacts 75% (n=15). 35% (n=7) reported significant improvement in pain after chemotherapy. Fear of death/treatment failure was expressed in 75% (n=15) but all reported feeling reassured after speaking to medical/nursing team. 100% (n=20) were highly satisfied with supports offered, including physiotherapy/psycho-oncology/dietician, MSW/cancer support centres. 90% (n=18) were dissatisfied with hospital meals (lack of variety, odour, timing). Based on these results the department of dietetics have engaged with catering and a specialised menu is now available in oncology. 10% (n=2) had specialist surgery in Birmingham, England and upon repatriation reported difficulty accessing rehabilitative programmes. 1 patient only was aware of the concept of survivorship. A patient/staff information day will be held on campus in July 2023 to improve awareness and understanding of sarcoma.

Conclusion: This survey is the first patient-reported outcome data in adult/AYA sarcoma in Ireland and will help to inform practice development in future. There is a significant lack of awareness of sarcoma and cancer survivorship in general in Ireland. A specialist ANP-led sarcoma survivorship clinic will be established in SVUH to help address this, and patient/public education initiatives are planned. We found that patients who have sarcoma surgery in the UK lack support for rehabilitation in Ireland, and ISG will focus on this as part of practice development. The investigators will continue to interview patients and an updated report will be presented at CTOS.

(P 157) QUALITY OF LIFE DURING TREATMENT FOR METASTATIC SARCOMA - FEASIBILITY OF ELECTRONIC PATIENT REPORTED OUTCOMES

Bodil E. Engelmann, MD, PhD[1]; Stine K. Christensen, B.Sc.[2]; Ninna Aggerholm-Pedersen, MD, MSc, PhD, Associate Professor[3]; Niels Junker, MD, PhD[1]; Anders Krarup-Hansen, N/A, MD, PhD, Associate Professor[1], (1) Department of Oncology, Copenhagen University Hospital, Herlev & Gentofte Hospital, Herlev, Hovedstaden, Denmark, (2) Copenhagen University Hospital at Herlev, Denmark, Herlev, Hovedstaden, Denmark, (3) Department of Oncology, Aarhus University Hospital, Aarhus, Midtjylland, Denmark

Objective: Patients with metastatic sarcoma have multiple complex symptoms. Systematic assessment of patient reported outcome (PRO) measures from patients with sarcoma provide insight into the effectiveness of care from the patient perspective. This is particularly valable information in rare cancers where PRO data from large randomized trials are scarce. In the Danish PRO Sarcoma project, electronic versions of validated PRO instruments are used to

capture data on health realted quality of life (HR-QoL) during sarcoma treatment, for use in daily practice as well as for quality assurance in the Danish Sarcoma Database. Patient self-report using electronic PRO instruments can itself improve symptom-management, communication, HRQoL and satisfaction with care. Furthermore, PRO measures can help identify patients that need supportive or rehabilitation interventions, and ultimately improve quality of life during treatment. In this feasibility study, we present PRO data from the PRO sarcoma project in a well defined group of patients with metastatic soft tissue sarcoma.

Methods: Patients 18 years of age or older with metastatic or locally advanced soft tissue sarcoma receiving palliative combination treatment with gemcitabin and dacarbazine at the Department of Clinical Oncology, Copenhagen University Hospital at Herlev, Denmark, between May 1st 2021 and March 28th 2023 were included in this retrospective study. All patients had the option to fill in Danish language versions of the EORTC QLQ C30 questionnaire which are sent electronically via an app (minSP by EPIC) connected to the patient´s electronic patient record (Sundhedsplatformen by EPIC). Questionnaires are accessible 7 days before the clinic appointment. Only clinic appointments with scan results and assessment of further treatment strategy are linked to PRO questionnaires. Clinical data as well as PRO data were collected from electronic patient records. Clinical characteristics of patients with and without reported PRO data are presented, as well as data on HR-QoL during treatment with gemcitabin and dacarbazine. Time to progression (TTP) and overall survial (OS) were assessed for the whole group using the Kaplan Meier model.

Results: 29 patients, 20 women and 9 men, received palliative treatment with gemcitabin and dacarbazine between May 1st 2021 and March 28th 2023. Most of the participants (27 out of 29) were diagnosed with leiomyosarcoma; two patients were treated for metastatic solitary fibrous tumour. Fifteen patients (11 women/4 men, median age 67 years) provided PRO data from one ore more EORTC QLQ C-30 questionnaire during treatment with gemcitabin and dacarbazine. There was no difference with regards to age, gender, sarcoma subtype, treatment line or treatment length between the patients that provided PRO data and the patients that did not provide any data. Median HR-QoL was 58,33 (range 31-100, N = 15) at the first assessment during treatment. At the second assessment, median QoL was 66,67 (range 44-83,33, N = 7). At the third assessment, median HR- QoL was 75 (range 50-91,67, N = 4). For two patients that provided four sets of PRO data, HR-QoL was 66,67 and 75. Median OS for the whole group was 1.56 years (95%CI: 1.13-2.45), median TTP 0.64 years (95%CI: 0.37-0.97).

Conclusion: This single center experience shows that electronic PRO capture in patients with metastatic sarcoma undergoing palliative treatment is feasible and shows substantial and clinically significant impairment of HR-QoL that calls for intervention. This data set does not provide any information about the reasons why almost half of the eligible patient choose not to answer the electronic PRO instruments, and no significant clinical differences between the groups of responders and non-responders are found. Survival data are comparable to data from the literature. Nationwide PRO capture as established in the Danish PRO Sarcoma project will provide further insight to HR-QoL for patients with sarcoma during and after treatment.

Medical & Pediatric Oncology and Trials

(P 158) SORAFENIB OR ANTHRACYCLINE-BASED CHEMOTHERAPY IN ADULT PATIENTS WITH PROGRESSIVE DESMOID TUMORS

Philippos A. Costa, MD[1]; Arshia Arora, MD[2]; Nipat Chuleerarux, MD[3]; Leticia Campoverde, MD[3]; Yanelys Fernandez, MD[3]; Baylee Bakkila, BS, BA[4]; Heng Tan, MD[3]; Priscila Barreto coelho, MD[3]; Andrea P. Espejo-Freire, MD[5]; Gina Z. D'Amato, MD[6]; Emily Jonczak, MD[7]; Ty Subhawong, MD[3]; Hari Deshpande, MD[1]; Kelly Olino, MD[4]; Jonathan Trent, MD[3], (1) Yale University, Hamden, CT, (2) University of Miami, Florida, FL, (3) University of Miami, Miami, FL, (4) Yale University, New Haven, CT, (5) University of Minnesota, MInneapolis, MN, (6) Sylvester Comprehensive Cancer Center, Miami, FL, (7) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL

Objective: Desmoid tumors are soft tissue neoplasms that, although unable to metastasize, can cause significant morbidity due to local infiltration. Occasionally, they may lead to death due to fast growth compromising vital structures. In this context, a more robust and timely response is required. Although the therapeutic arsenal for desmoid tumors is growing, no randomized controlled trials compare the efficacy of different systemic therapies. Here we aim to compare anthracycline-based regimens and sorafenib to assess efficacy, response rates, and time to response.

Methods: We conducted a retrospective comparative analysis across three academic sites to evaluate the outcomes of patients with desmoid tumors who received either sorafenib or anthracycline-based therapy over a one-year period. The primary endpoint was investigator-assessed progression-free survival. The secondary endpoints were objective response rates (assessed using RECIST 1.1 criteria), time to response, and adverse events (graded according to CTCAE). Kaplan-Meier and log-rank tests were used to estimate progression-free survival and compare time to response.

Results: From 2005 through 2022, a total of 54 patients received sorafenib and 38 anthracycline-based therapy. Baseline characteristics for both groups are detailed in Table 1. The 1-year progress-free survival rate was 61% (95% confidence interval [CI], 43 to 79) for the anthracycline-based group and 59% (95% CI, 44 to 74) for the sorafenib group, which was not statistically different ($p = 0.75$), which remained not different in pre-specified subgroup analyses (sex, tumor location, previous surgery, and previous systemic therapy). The percentage of patients who had an objective response at 1 year was 34% with anthracycline-based therapies and 19% with sorafenib ($p=0.34$), with a median time to response of 4 months on the anthracycline-based group and 6.5 months on the sorafenib. In the sorafenib group, the most frequently reported adverse events were grade 1 or 2 events of hand-foot syndrome (40%), fatigue (26%), diarrhea (22%), and hypertension (13%). In the anthracycline-based group, grade 1 or 2 events of nausea (34%), rash (23%), fatigue (18%), and mucositis (13%). G3 events were higher in the anthracycline-based group, 26% vs. 9% ($p < 0.05$), although only 18% of patients discontinued therapy due to side effects in the anthracycline-based group compared to 24% in the sorafenib group.

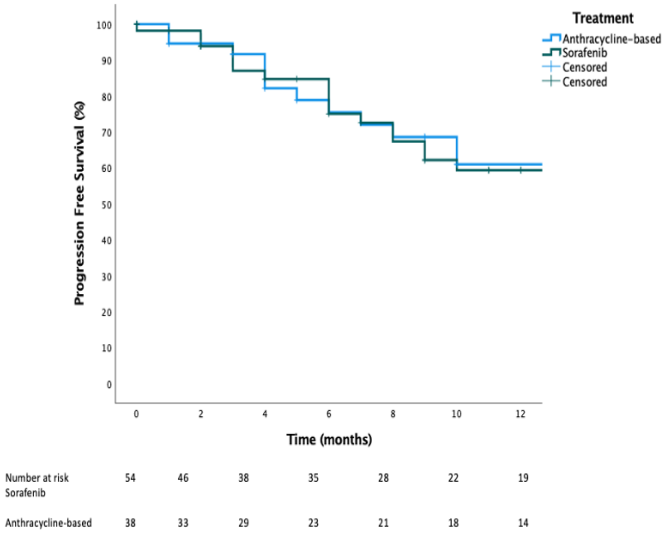
Conclusion: Among patients with progressive or symptomatic desmoid tumors, anthracycline-based therapy and sorafenib did not show statistically significant differences in progression-free survival rates, response rate, or time to response. An important limitation of the findings is the

small group sizes and no centralized radiological review, which may lead to inter-reader variability during RECIST evaluation.

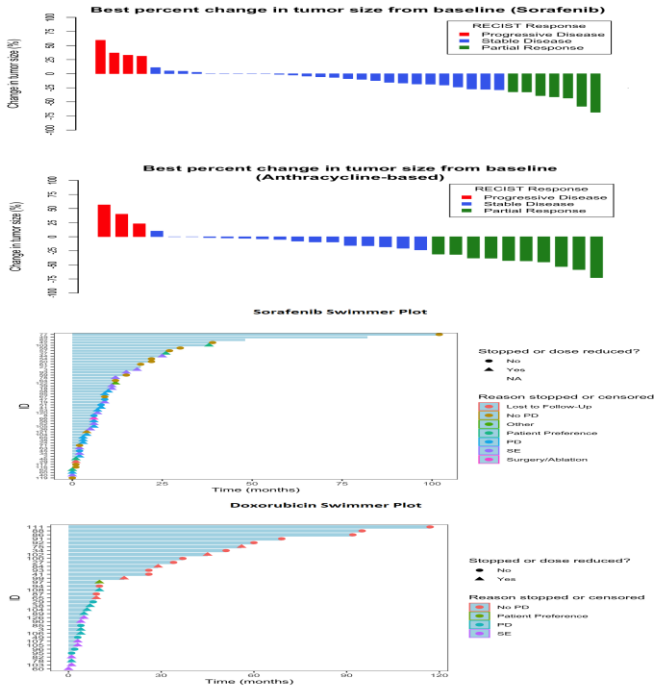
Table 1. Demographic and Clinical Characteristics

	Sorafenib	Anthracycline-based
Median age (range)--yr	36 (5-77)	35 (9-71)
Sex-- no.(%)		
Female	39 (72.2)	24 (63)
Male	15 (27.2)	14 (37)
Primary Tumor site-- no.(%)		
Intra-abdominal	4 (7.4)	6 (15)
Extra-abdominal	50 (92.6)	32 (85)
Largest tumor size--cm	9.0 (1.8-23.0)	9.8 (2-27)
Familial Polyposis-- no.(%)	2 (3.7)	2 (5.2)
Previous surgical resection-- no.(%)	11 (20.3)	9 (23.6)
Previous cryoablation—no.(%)	6 (11.1)	3 (7.8)
Previous radiation therapy-- no.(%)	5 (9.2)	2 (5.2)
Previous systemic therapy-- no.(%)	19 (35.1)	14 (36.8)
Number of lines of therapy — Median(range)	1 (1-5)	1 (1-5)
Choice of therapy		
Doxorubicin -- no.(%)	NA	18 (47.3)
Doxorubicin dacarbazine -- no.(%)	NA	19 (50)
Other	NA	1 (2.6)
Sorafenib	54 (100)	

Figure 1. Kaplan–Meier Estimates of the Duration of Progression-free Survival.



Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, and clinical progression were used by the investigators to identify disease progression. Data from patients who did not have progression were censored and marked by a tick.



(P 159) IN BETWEEN OF LYMPHOMAS AND SARCOMAS: WHERE DO HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS (HDCN) STAND? A RETROSPECTIVE, MULTI-INSTITUTIONAL STUDY FROM THE ITALIAN SARCOMA GROUP (ISG)

Anna Maria Frezza, MD[1]; Luisa Lorenzi, MD[2]; Giacomo Giulio Baldi, MD[3]; Giuseppe Badalamenti, MD[4]; Alfredo Berruti, MD[5]; Antonella Brunello, MD, PhD[6]; Paolo Giovanni G. Casali, MD[7]; Lorenzo D'Ambrosio, MD[8]; Ida De Luca, MD[9]; Angelo Paolo Dei Tos, N/A, MD[10]; Pierluigi Di Mauro, MD[11]; Claudia Giani, MD[12]; Alessandro Mazzocca, MD[13]; Alessandra Merlini, MD, PhD[14]; Carlo Morosi, MD[15]; Elena Palassini, N/A, MD[16]; Stefano Radaelli, MD[17]; Claudia Sangalli, MD[18]; Marta Sbaraglia, MD[19]; Bruno Vincenzi, MD, PhD[13]; Fabio Facchetti, MD[20]; Alessandro Gronchi, MD, FSSO[21]; Silvia Stacchiotti, MD[1], (1) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (2) University of Brescia, Brescia, Lombardia, Italy, (3) Medical Oncology Department, "Santo Stefano" Hospital, Prato, Italy, Prato, Toscana, Italy, (4) Policlinico P. Giaccone, Palermo, Sicilia, Italy, (5) University of Brescia, ASST Spedali Civili, Brescia, Lombardia, Italy, (6) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy, Padua, Veneto, Italy, (7) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (8) University if Turin, Turin, Italy, Torino, Piemonte, Italy, (9) Policlinico P Giaccone, Palermo, Sicilia, Italy, (10) Department of Medicine, University of Padua School of Medicine, Padova, Italy, Padova, Veneto, Italy, (11) ASST Spedali Civili di Brescia, Brescia, Lombardia, Italy, (12) Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (13) Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Lazio, Italy, (14) Department of Oncology, University of Turin, Torino, Piemonte, Italy, (15) Department of Radiology, Fondazione IRCSS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (16) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (17)

Sarcoma Service, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (18) Radiation Oncology Department, Fondazione IRCCS Istituto Nazionale Tumori, Milano, Lombardia, Italy, (19) Department of Pathology, Azienda Ospedale-Università Padova, Padova, Italy, Padova, Veneto, Italy, (20) Pathology Unit, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Lombardia, Italy, (21) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy

Objective: HDCNs encompass heterogeneous, ultra-rare neoplasms, arising from cells of haematological or mesenchymal derivation. Consistently, their clinical behaviour recognises features of both haematological cancers and soft tissue sarcomas (STS), with many unresolved questions on the best treatment strategy.

Methods: In the framework of the ISG, we retrospectively reviewed all consecutive cases of HDCNs (histiocytic sarcoma, HS; Langerhans cell sarcoma, LC-S; interdigitating dendritic cell sarcoma, IDC-S; indeterminate dendritic cell tumour, IDC-T; follicular dendritic cell sarcoma, FDC-S; fibroblastic reticular cell tumour, FRC-T) patients (pts) diagnosed between 2000 and 2022 in 7 centers. All diagnoses were confirmed by dedicated pathologists according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (2017). Radiological response was assessed retrospectively using RECIST1.1 threshold for complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD). Median (m) follow up (FUP) and overall survival (OS, defined as interval between diagnosis and death by any cause) were computed by Kaplan-Meier.

Results: Overall, 56 consecutive HDCNs pts were identified and included in the study (characteristics in table 1): 34 presented with local disease (LD; 7 HS, 17 FDC-S, 3 LC-S, 3 IDC-S, 4 FRC-T), 3 with locally advanced disease (LAD; 1 HS, 1 LC-S, 1 IDC-S), 19 with distant metastases (DM; 7 HS, 6 FDC-S, 1 LC-S, 2 IDC-S, 2 FRC-T, 1 IDC-T). The m-FUP was 86 mos, with a 5-yr OS estimate of 0.97 in pts with LD and 0.5 in pts with LAD or MD.
For pts with LD, all HS underwent surgery with radical intent, 1/7 adjuvant (adj) radiotherapy (RT, 56 Gy), 2/7 adj chemotherapy (CT). The 5-yr OS estimate was 0.87: 1 pt developed local recurrence (LR), 2 DM. All FDC-S pts underwent surgery, 7/17 adj RT (range: 50-66 Gy) and 4/17 adj CT. The 5-year OS estimate was 0.94: 2 pts developed LR, 3 DM. Similarly, all patients with LC-S, IDC-S, FRC-T underwent surgery, 2 pt (LC-S, IDC-S) adj RT (60 Gy, 56 Gy), none CT. None of them recurred. Regardless the HDCNs type, all pts recurring locally did not receive adj RT nor those who developed DM received adj CT (details in table 2).
For pts with MD, the 5-yr OS estimate in HS was 0.50, in FRC-T, IDC-S, IDC-T 0.8. In FDC-S the m-OS was 8 mos, with all pts dead at last FUP. The LC patient died of a B-lymphoma after LC CR on CT.
All pts (4) managed with sequential, definitive anthracycline-based CT and RT (range: 30-55 Gy) are alive and achieved prolonged CR: 2 pts (HS and LC-S) treated for LAD (figure 1) are ADF at 6 and 3 yrs, respectively; 1 patient with a thoracic LR of FDC-S and 1 pt with HS of the gluteus with a single paravertebral metastasis are ADF at 1 and 7 yrs, respectively.
As for CT activity, overall, 20 pts evaluable for response received anthracycline-based CT (LD, LAD, MD included), achieving as best response CR in 7/20 (35%), PR in 6/20 (30%), SD in 4/20 (20%), PD in 3/20 (15%), with a global ORR of 65%. In FDC-S and HS, the ORR was 34% and 78% respectively. Details on response to systemic regimens are reported in table 3.

Conclusion: In this series, the outcome in primary, resected HDCNs is favorable, with an estimated 5-yr OS higher than 80%. Advanced FDC-S remains an unmet need with a m-OS < 12 mos. Similarly to what reported in lymphomas, long lasting CR were achieved in different HDCN types treated with definitive CT/RT, which can be an option for cases unmanageable with surgery. Also, anthracyclines showed a relevant activity in HDCNs, especially in HS, with

an ORR superior to those observed in STS. Taken together, these data suggest a possible role for definitive CT/RT and perioperative CT and RT in LD, which needs to be confirmed in a larger series.

Table 1. Population characteristics

	H-S	FDC-S	LC-S	IDC-S	FRC-T	IDC-T
Patients	15	23	5	6	6	1
Demographics						
Age at diagnosis, mean (range)	55 (38-71)	55 (30-84)	55 (30-84)	63 (43-72)	52 (51-71)	
M/F ratio	11/4	13/10	2/3	2/4	4/2	
Systemic symptoms at baseline						
Yes	3 (20%)	8 (35%)	2 (40%)	0	1 (17%)	
No	12 (80%)	15 (65%)	3 (60%)	6 (100%)	5 (83%)	
History of hematological neoplasms						
Yes	2 (13%)	1 (4%)	2 (40%)	0	0	
No	13 (87%)	22 (96%)	3 (60%)	6 (100%)	6 (100%)	
Disease stage at baseline						
Localized	7 (47%)	17 (74%)	3 (60%)	3 (50%)	4 (67%)	-
Locally advanced	1* (6%)	-	1 (20%)	1 (17%)	-	-
Metastatic	7 (47%)	6 (26%)	1 (20%)	2 (33%)	2 (33%)	1
Disease extent (metastatic pts)						
Nodal-only	4/7	3/6	1/1	0	2/2	-
Locoregional	2	-	-	-	2	-
Extraregional	2	3	1	-	-	-
Extranodal-only	2/7	1/6	0	0	0	-
Both	1/7	2/6	0	2/2	0	1
Treatment (localized disease)						
Surgery	7/7	17/17	3/3	3/3	4/4	-
Radiation therapy	2/7	7/17	2/3	0	0	-
Preoperative	0	1	0	-	-	-
Postoperative	1	6	2	-	-	-
Definitive	0	0	0	-	-	-
Chemotherapy	2/7	4/17	0	0	1/4	-
Preoperative	1	4	0	-	-	-
Postoperative	1	0	0	-	1	-
Sequential with RT	0	0	1	-	-	-
Treatment (locally advanced and metastatic disease)					2/2	-
Surgery	6/8	1/6	0/2	2/3	2	-
Primary	4	1	-	1*	2	-
Metastases	2	0	-	1	0/2	1
Radiation therapy	2/8	1/6	1/2	2/3	-	1
Palliative	0	1	-	1	-	-
Definitive	2	0	1	1*	1/2	1
Chemotherapy	8/8	5/6	2/2	2/3	1	1
Palliative	6	5	1	1	-	-
Sequential with RT	2	0	1	1*	0	0
Best supportive care	0	1/6	0	0		

*Locally advanced patient treated with preoperative RT, surgery, post-operative CT

Table 2. Outcome of primary, localized patients undergoing surgery with radical intent

	H-S	FDC-S	LC-S	IDC-S	FRC-T	
Patients with LD	7	17	3	3	4	
Local recurrence	1	2	0	0	0	HS:
Adj RT (primary) yes	0	0	-	-	-	LR 6 mos after R1 surgery, new surgery, ADF at 1 yr
Adj RT (primary) no	0	0	-	-	-	DM 30 mos after R0 surgery, CT, AWD
Distant metastases	2	3	0	0	0	DM 5 mos after R0 surgery, CT, DOD
Adj CT (primary) yes	0	0	-	-	-	
Adj CT (primary) no	0	0	-	-	-	FDC-S:
Disease specific death	1	1	0	0	0	LR 26 mos after R1 surgery, definitive CT/RT, ADF at 1 yr
Adj CT (primary) yes	0	0	-	-	-	LR 12 mos after R0 surgery, new surgery, ADF at 22 mos
Adj CT (primary) no	0	0	-	-	-	DM 21 mos after R0 surgery, CT, AWD
						DM 3 mos after R0 surgery, CT, DOD
						DM (single) 4 yr after R0 surgery, definitive RT, ADF at 6 yrs

LD: localized disease; RT: radiation therapy; CT chemotherapy; Adj: adjuvant; ADF: alive and disease free; AWD alive with disease; DOD: dead of disease

Table 3. Radiological response to medical therapies

	H-S	FDC-S	LC-S	IDC-S	FRC-T	IDC-T
Anthracycline-based regimens*						
Patients (evaluable for response)	10 (9**)	6 (6)	2(2)	2(1**)	2(1**)	1(1)
CR	3/9 (33%)	1/6 (17%)	1	1	0	1
PR	4/9 (44%)	1/6 (17%)	1	0	0	0
SD	2/9 (23%)	1/6 (17%)	0	0	1	0
PD	0	3/6 (49%)	0	0	0	0
High-dose ifosfamide						
Patients (evaluable for response)	-	-	-	-	-	-
CR						
PR						
SD						
PD						
Ifosfamide, etoposide +/- cisplatin						
Patients (evaluable for response)	1 (1)	-	-	-	-	-
CR						
PR	1					
SD						
PD						
Cyclophosphamide +/- vincristin						
Patients (evaluable for response)	1 (1)	2 (2)	-	-	-	-
CR						
PR	1					
SD		2 (2)				
PD						
Gemcitabine +/+ docetaxel						
Patients (evaluable for response)	-	1 (2)	-	-	-	-
CR						
PR		1				
SD						
PD						
Pazopanib						
Patients (evaluable for response)	1 (1)	1 (2)	-	-	-	-
CR						
PR						
SD		1				
PD	1					

*Anthracycline-based regimens included anthracycline, anthracycline + ifosfamide (or cyclophosphamide), anthracycline + cisplatin, CHOP +/- etoposide, COMP

**1 HS, 1 IDC-S, 1 FRC-T not evaluable for response as treated with post-operative CT

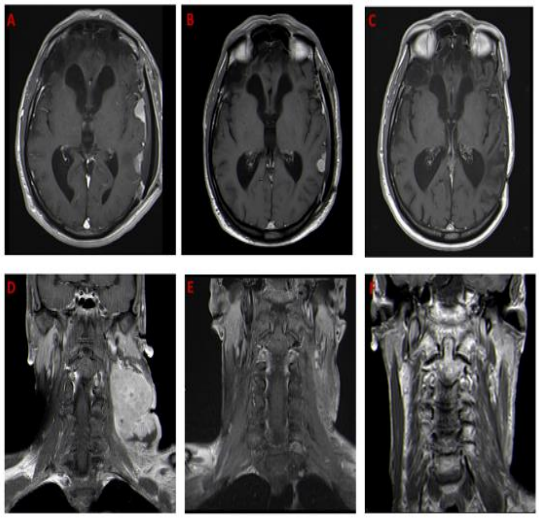


Figure 1. Radiologic complete response to definitive, sequential anthracycline-based therapy and radiation therapy

A) Post contrast T1-weighted magnetic resonance imaging (MRI), axial view, showing a 48yr-old pt with multifocal meningeal HS treated with 4 cycles of doxorubicin and cyclophosphamide with PR (B) and definitive radiation therapy (44 Gy) with CR (C), currently progression free after 6 yrs. (D) post T1-weighted magnetic resonance imaging (MRI), coronal view, showing a 51-yr-old pt with left laterocervical and sovraclavicular lymph nodal LC-S treated with 6 cycles of doxorubicin and ifosfamide with PR (E) and definitive radiation therapy (55 Gy) with CR (F), currently progression at 3 yrs

(P 160) A PROSPECTIVE STUDY EVALUATING THE ASSOCIATION OF PATIENT-REPORTED OUTCOMES WITH ACTIVITY LEVEL AND SLEEP PATTERNS IN SARCOMA SURVIVORS

Elizabeth J. Davis, MD[1]; Emma A. Schremp, MS[1]; Tatsuki Koyama, PhD[1]; Lili Sun, PhD, MS[1]; Lauren King, MPH[2]; Vicki L. Keedy, MD, MSCI, FASCO[1]; Michael J. Robinson, MD[1]; Eric Shinohara, MD, MSCI[1]; Tuya Pal, MD[1]; Ben H. Park, MD, PhD[1]; Xiao-Ou Shu, MD, PhD[1]; Debra L. Friedman, MD, MS[1]; Scott Borinstein, MD, PhD[1], (1) Vanderbilt University Medical Center, Nashville, TN, (2) Vanderbilt University Medical Center, Greenbrier, TN

Objective: Sarcomas are a rare heterogeneous malignancy group affecting both sexes and all ages. Treatment is often intensive, resulting in long-term toxicities. Given the rarity of sarcoma, there is a paucity of data defining physical and emotional outcomes. The Cohort to Augment the Understanding of Sarcoma Survivorship Across the Lifespan (CAUSAL) was constructed at Vanderbilt University Medical Center (VUMC) to evaluate health-related outcomes.

Methods: Sarcoma patients treated at VUMC from 2012 – present are enrolled into CAUSAL. Patient demographics, tumor and treatment data are abstracted from the electronic medical record. Participants complete surveys including PROMIS-57 consisting of seven domains including physical function, sleep disturbance, fatigue, and satisfaction with participation in social roles, which are reported here. Participants who have completed treatment are given a FitBit to track activity level and sleep over 12 weeks. Data are synced to an online platform, Fitabase. Body Mass Index (BMI) is assessed at study entry. For each participant, daily step count, active minutes, and sleep minutes were summarized using medians. To estimate the association between PROMIS-57 scores and activity and sleep data (FitBit), linear regression models were fit controlling for age, sex, and BMI of participants.

Results: Of 736 CAUSAL participants, 421 completed the PROMIS-57. Of those, 111 participants also had FitBit data and are included in this analysis. Median step count was 6626 (quartiles: 4436, 8744). For reference, the average FitBit step count data for the US population is 8170. Median daily activity was 260 minutes (quartiles: 209.5, 317.5). Median duration of sleep was 449 minutes (quartiles: 400, 475) which was greater than the average reported by FitBit for the general US population (436 min). Linear regression analysis determined that step count was significantly associated with PROMIS physical function score ($p < 0.001$). A 10-point increase in physical function score is associated with an increase of 1393 (95% CI: 789, 1997) steps when adjusting for age and sex. Active daily minutes were significantly inversely correlated with PROMIS fatigue score ($p = 0.012$). A 10-point increase in fatigue score is associated with an average decrease of 20 (95% CI: 36, 45) minutes when adjusting for age and sex. Compared with participants who received treatment within 30 days prior to CAUSAL enrollment, participants not receiving treatment had 5.38 (95% CI: 2.96, 7.8) higher physical function score and 6.26 (95% CI: 2.81, 9.71) higher satisfaction in their social roles. There was no significant association between minutes of sleep recorded by FitBit and PROMIS sleep disturbance.

Conclusion: Using PROMIS-57, and FitBits, we demonstrated a positive association between higher step counts and self-reported higher physical function, in addition to a negative association between FitBit recorded activity level and fatigue score. We did not find a significant association between FitBit recorded sleep and self-reported sleep disturbance. Furthermore, our data suggest patients with sarcoma sleep more and walk less than the general US population. As enrollment continues in CAUSAL, we will focus on PROMIS scores over time

and their association with activity level and sleep.

(P 161) EFFICACY, SAFETY, AND TREATMENT HOLD WAIT-AND-SEE OUTCOMES OF LAROTRECTINIB IN PEDIATRIC PATIENTS WITH TRK FUSION SARCOMAS

Catherine M. Albert, MD[1]; Cornelis M. van Tilburg, MD, PhD[2]; Daniel Orbach, MD[3]; Miranda Dierselhuis, MD, PhD[4]; Claudia Blattmann, MD[5]; Birgit Geoerger, MD, PhD[6]; Steven G. Dubois, MD, MS[7]; Noah Federman, MD[8]; Ramamoorthy Nagasubramanian, MD[9]; Tanya C. Watt, MD[10]; Ricarda Norenberg, MSc[11]; Esther De La Cuesta, MD, MBA[12]; Vadim Bernard-Gauthier, PhD[13]; Leo Mascarenhas, MD, MS[14]; Theodore W. Laetsch, MD[15]; Yizhuo Zhang, MD, PhD[16], (1) Seattle Children's Hospital, University of Washington, Fred Hutchinson Cancer Center,, Seattle, WA, (2) Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg University Hospital and German Cancer Research Center (DKFZ),, Heidelberg, Baden-Wurttemberg, Germany, (3) SIREDO Oncology Center (Care, Innovation and Research for Children and AYA with Cancer), Institut Curie, and University PSL, Paris, Ile-de-France, France, (4) Prinses Maxima Centrum, Utecht, Utrecht, Netherlands, (5) Olgahospital, Stuttgart, Baden-Wurttemberg, Germany, (6) Gustave Roussy Cancer Center, Université Paris-Saclay, INSERM U1015, Villejuif, Ile-de-France, France, (7) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA, (8) David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, (9) Nemours Children's Hospital, Orlando, FL, (10) University of Texas Southwestern Medical Center, Dallas, TX, (11) Chrestos Concept GmbH & Co. KG, Essen, Rheinland-Pfalz, Germany, (12) Bayer HealthCare Pharmaceuticals, Inc., Whippany, NJ, (13) Bayer Pharmaceuticals, Inc., Toronto, ON, Canada, (14) Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, (15) Children's Hospital of Philadelphia/University of Pennsylvania, Philadelphia, PA, (16) Sun Yat-sen University Cancer Center, Guangzhou, Guangdong, China (People's Republic)

Objective: Neurotrophic tyrosine receptor kinase (NTRK) gene fusions are oncogenic drivers in a diverse range of tumor types. Larotrectinib is a highly selective, CNS-active tropomyosin receptor kinase (TRK) inhibitor approved for the treatment of pediatric and adult patients (pts) with TRK fusion cancer. We report updated larotrectinib efficacy and safety data in pediatric pts with TRK fusion sarcomas, and outcomes in the subset of pts who stopped larotrectinib in the absence of disease progression.

Methods: Pediatric pts (< 18 years old) with TRK fusion sarcomas treated with larotrectinib were identified from two clinical trials (NCT02576431, NCT02637687 [SCOUT]). Most pts received larotrectinib 100 mg/m2 (maximum dose of 100 mg) twice daily. Responses were assessed by an independent review committee (IRC) per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1). Pts in the SCOUT study were permitted to stop larotrectinib in the absence of on-treatment disease progression (wait-and-see); pts who stopped treatment for ≥28 days were actively followed for progression according to protocol for a post-hoc analysis. If re-treated due to progression, response was re-assessed by investigator per RECIST v1.1.

Results: As of July 20, 2022, 89 pediatric pts with TRK fusion sarcomas were included: 49 (55%) infantile fibrosarcoma (IFS), 39 (44%) other soft tissue sarcomas (STS), and one (1%) bone sarcoma. Median age at enrollment was 2.2 years (range 0–18). Thirty-seven (42%) pts had prior surgery and 61 (69%) had prior systemic therapy. Testing methods are shown in

Table 1. Among 88 IRC-eligible pts, the objective response rate was 85% (95% confidence interval [CI] 76–92; Table 2), including 43 pts (49%) with complete response (CR) or pathological CR (pCR). Median time to response was 1.8 months (mo; range 0.9–5.4). Median duration of response, progression-free survival, and overall survival are shown in Table 2. Treatment duration ranged from 0+ to 75+ mo. At data cutoff, 58 (66%) pts were still on treatment or in wait-and-see. Thirty (34%) pts had permanently discontinued treatment, with 16 discontinuing due to tumor progression. Treatment-related adverse events (TRAEs) were mostly Grade 1/2. Grade 3/4 TRAEs occurred in 31 (35%) pts. Four (5%) pts discontinued due to a TRAE.
For the post-hoc analysis, 44 pts stopped larotrectinib in the absence of on-treatment progression: 28 (64%) with IFS and 16 (36%) with other STS. Median time to discontinuation in these pts was 14.6 mo (range 3.0–64.6). Twenty-three (52%) pts discontinued after achieving CR (including 9 with pCR), 18 (41%) with partial response (PR), and three (7%) with stable disease (SD). Of these 44 pts, 15 (34%) who stopped treatment had documented subsequent progression (Table 3). Median time from discontinuation to subsequent progression was not reached at the time of data cutoff; however, the range from discontinuation to subsequent progression for these 15 pts was 0.9–15.3 mo. Fourteen pts resumed treatment after documented progression. Nine of these 14 pts showed re-response to treatment (five CR, four PR); four pts had SD and one had no post-resumption disease assessments. All 44 pts were alive at data cutoff.

Conclusion: Larotrectinib demonstrated robust and durable responses, extended survival benefit, and a favorable safety profile in pediatric pts with TRK fusion sarcomas. These data support the wider adoption of next-generation sequencing panels that include NTRK gene fusions to identify pts who may benefit from treatment. In the post-hoc analysis, 15 of 44 pts who stopped treatment had documented subsequent progression. The majority of patients that progressed derived clinical benefit from retreatment with larotrectinib with 64% experiencing an objective response. The post-hoc analysis suggests that treatment discontinuation is feasible, with possibility to resume therapy as needed. Longer follow-up is required to better determine which pts could be candidates for treatment discontinuation.

Table 1. Testing methods used to identify *NTRK* gene fusions

Testing methods	Patients (N=89), n
NGS	60
PCR†	13
FISH	13
Chromosomal microarray	1
NanoString	1
Sequencing 5′RACE	1

†Includes 12 RT-PCR and one nested PCR. FISH, fluorescence in situ hybridization; NGS, next-generation sequencing; *NTRK*, neurotrophic tyrosine receptor kinase; PCR, polymerase chain reaction; RACE, rapid amplification of copy DNA ends; RT, reverse transcriptase.

Table 2. Response to treatment

	IFS (n=45)	Other STS (n=35)	All pediatric sarcomas (N=89)‡
IRC-eligible patients, n	49	38	88‡
ORR, % (95% CI)	92 (80–98)	79 (63–90)	85 (76–92)
Best overall response, n (%)			
Complete response	21 (43)	10 (26)	31 (35)
Pathological complete response†	7 (14)	5 (13)	12 (14)
Partial response	17 (35)	15 (39)	32 (36)
Stable disease	4 (8)	4 (11)	8 (9)
Progressive disease	0	2 (5)	3 (3)‡
Not evaluable	0	2 (5)	2 (2)
Median DoR, % (95% CI)	38.4 (22.0–NE)	34.5 (17.5–NE)	34.5 (23.4–NE)
Median follow-up for DoR, months	37.3	30.9	31.3
24-month DoR rate, % (95% CI)	60 (44–77)	69 (48–90)	64 (51–77)
Median PFS, % (95% CI)	40.2 (23.9–NE)	28.0 (18.8–NE)	33.3 (23.9–48.7)
Median follow-up for PFS, months	39.1	32.5	32.9
24-month PFS rate, % (95% CI)	66 (51–81)	60 (39–80)	63 (51–75)
Median OS, % (95% CI)	Not reached	Not reached	Not reached
Median follow-up for OS, months	42.8	37.3	41.3
48-month OS rate, % (95% CI)	98 (93–100)	78 (64–93)	89 (82–96)

†Pathological complete response is defined as no pathologic evidence of tumor, negative surgical margins, and no other evidence of disease. ‡Includes one patient with bone sarcoma who had progressive disease as best overall response.

CI, confidence interval; DoR, duration of response; IFS, infantile fibrosarcoma; IRC, independent review committee; NE, not estimable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; STS, soft tissue sarcoma.

Table 3. Progression after discontinuing treatment by surgery status

	Surgical (n=20)					Non-surgical (n=24)	Total (N=44)
Local status at the end of larotrectinib, (n)	pCR (7)	Unknown (1)	R0 (5)	R1 (6)	R2 (1)		
Progressed, n	2	0	0	1	1	11	15

pCR, pathological complete response; R0, no residual tumor; R1, microscopic residual tumor; R2, macroscopic residual tumor.

(P 162) LONG TERM SAFETY AND EFFICACY OF COMBINED DOXORUBICIN-IFOSFAMIDE CHEMOTHERAPY WITH PREOPERATIVE HYPOFRACTIONATED RADIOTHERAPY IN MARGINALLY RESECTABLE SOFT TISSUE SARCOMAS

Anna M. Czarnecka, MD PhD[1]; Piotr Blonski[1]; Maria Krotewicz, MD[1]; Mateusz Jacek Spalek, MD, PhD[2]; Iga Płachta, MD[1]; Klaudia Bobak, MSc[3]; Aneta Borkowska, MD PhD[1]; Michal Wagrodzki, MD, PhD[4]; Hanna Koseła-Paterczyk, MD PhD[1]; Piotr L. Rutkowski, MD, PhD[5], (1) Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (2) Maria Sklodowska-Curie National Research Institute of Oncology, Department of Soft Tissue/Bone Sarcoma and Melanoma, Department of Radiotherapy I, Warsaw Poland , Warsaw, Mazowieckie, Poland, (3) Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, WArsaw, Mazowieckie, Poland, (4) Maria Sklodowska-Curie National Research Institute of Pathology, Warsaw Poland, Warsaw, Mazowieckie, Poland, (5) Maria Sklodowska-Curie National Research Institute of Oncology, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw Poland, Warsaw, Mazowieckie, Poland

Objective: There is no consensus on most efficient treatment for marginally resectable soft tissue sarcomas (STS) of the extremities and trunk wall to achieve the highest number of R0 resections. Based on our phase II UN-RESARC trial we implemented multidisciplinary treatment where patient received 5x5 Gy radiotherapy combined with doxorubicin-ifosfamide chemotherapy. Here we describe the long-term safety of this treatment approach along with the ratio of conservative surgeries with negative microscopic margins (R0).

Methods: The patients with marginally resectable STS were treated within single-arm prospective clinical trial and routine clinical practice according to the same regimen afterwards. The patients received the first course of AI chemotherapy - doxorubicin 75 mg/sqm and ifosfamide 10 g/sqm followed by 5x5 Gy radiotherapy and two additional courses of AI chemotherapy in the gap between radiotherapy and delayed surgery. Surgery was planned after 9 weeks of neoadjuvant chemo-radiotherapy. Adult patients were treated with radical intent. The primary endpoint was to assess the number of patients in whom R0 en-bloc radical resection was achieved. The secondary endpoint was to describe the safety of treatment. Patients treated with other chemoregimens, fractionation regimens, or in a palliative setting were not eligible for analysis. Common Terminology Criteria for Adverse Events (CTCAE) 4.0 was used to describe toxicity. Descriptive statistics were used to summarize the demographic and clinical characteristics. Median follow-up was estimated by Kaplan-Meier analysis with the reversed meaning of status indicator. Relapse-free survival (RFS) was calculated from the day of resection to the last follow-up, death without relapse, confirmed local relapse, or distant metastases. Clinical local recurrence was confirmed with CT or MRI and biopsy. Overall survival (OS) was calculated from the date of surgery to the last follow-up or death. Data cut-off was 24th June 2023. All statistical analyses were performed using R version 4.2.3.

Results: Eighty-two patients with median age of 58 years (25-76) met the eligibility criteria. Six patients had resectable lung metastases at baseline. Most common sarcoma subtypes diagnosed in these patients were undifferentiated pleomorphic sarcoma (n=33; 40.2%), myxofibrosarcoma (n=15; 18.3%), and malignant peripheral nerve sheath tumor (n=9; 11%). Median tumor diameter was 14.85 cm. 43% of patients presented with anemia of chronic disease. R0 resections were achieved in 57 (69.5%) patients while only 3 (3.7%) patients had to undergo limb amputations. Surgery-related adverse events according to Clavien-Dindo classification were reported in 8 (10%) patients in grade 3a and in 2 (2.4%) patients in grade 3b. Systemic therapy-related adverse events of grade ≥3 were reported in 39 (47.5%) patients,

with most common lymphopenia in 29 (35%) patients, neutropenia in 10 (12%), and febrile neutropenia in 4 (4.9%) patients. Due to toxicity doxorubicin dose was reduced in 19 (23.2%) patients and 4 (4.9%) patients received only one cycle of chemotherapy. No life-threatening complications were reported after surgery. Wound dehiscence occurred in 16 (20%) cases, but severe bleeding only in 3 (3.7%) patients. In total 10 (12%) patients required reoperation. Wound complications as per O'Sullivan criteria occurred in 22 (27%) patients. Nine patients (11%) experienced local recurrence. The median RFS was 20.8 (10.9 - 40) months with 43% of patients free of recurrence at 3 years. Median follow-up was 34.2 months, and median OS was not reached (3-year OS rate was 58%); while the longest overall survival was 81.6 months. None of the patients died before the surgery.

Conclusion: Although preoperative radiotherapy is usually associated with a higher risk of wound complications than postoperative radiotherapy and there is a fear of anthracycline administration with radiotherapy, hypofractionated radiotherapy combined with AI chemotherapy allows safe and efficient surgery in patients with marginally resectable STS.

Systemic therapy-related adverse events	
Total number of patients	82 (100%)
Any adverse event, grade ≥3	
Yes	39 (47.5%)
No	43 (52.5%)
Most common grade ≥3 adverse events	
Lymphocytopenia	29 (35%)
Anaemia	15 (18%)
Neutropenia	10 (12%)
Including febrile neutropenia	4 (4.9%)
Thrombocytopenia	2 (2.4%)
Was dose reduction required?	
No	59 (72%)
Yes, there was a single dose reduction	17 (20.7%)
Yes, but after single reduction, further reduction was required	2 (2.4%)
Patient received only one cycle of chemotherapy	4 (4.9%)
Surgery-related adverse events	
According to Clavien and Dindo	
None	53 (65%)
Grade 1	13 (16%)
Grade 2	6 (7%)
Grade 3a	8 (10%)
Grade 3b	2 (2.4%)
Most common surgical complications	
Wound dehiscence	16 (20%)
Wound infection	11 (13%)
Wound haematoma	4 (4.9%)
Severe haemorrhage	3 (3.7%)
Reoperation required	10 (12%)
Wound complications	
According to CTCAE	
None	56 (68%)
Grade 1	10 (12%)
Grade 2	8 (10%)
Grade 3	7 (9%)
Grade 4	1 (1.2%)
According to O'Sullivan	
No	60 (73%)
Yes	22 (27%)

(P 163) SELUMETINIB IN JAPANESE PEDIATRIC PATIENTS WITH SYMPTOMATIC INOPERABLE PLEXIFORM NEUROFIBROMA IN NEUROFIBROMATOSIS TYPE 1

Yoshihiro Nishida, MD, PhD; Norie Nonobe, MD, PhD; Hiroyuki Kidokoro, MD, PhD; Taichi Kato, MD, PhD; Kunihiro Ikuta, MD, PhD; Hiroshi Urakawa, MD, PhD; Tomohisa Sakai, MD, PhD; Hiroshi Koike, MD, PhD; Takeo Fujito, MD, PhD; Shiro Imagama, MD, PhD, Nagoya University Hospital, Nagoya, Aichi, Japan

Objective: A lifetime risk of developing malignant peripheral nerve sheath tumor (MPNST) in patients with neurofibromatosis type 1 has been reported as high as 15%. Unlike sporadic MPNST, MPNST in NF1 patients develops with malignant transformation of plexiform

neurofibroma (PN). It has also been reported that malignant transformation of PN to MPNST in NF1 patients develops through an intermediate tumor (ANNUBP). Because wide excision is the only curative treatment for MPNST, early detection and operation are extremely important. Selumetinib was allowed to use for patients with symptomatic, inoperable PN in children of neurofibromatosis type 1 (NF1) from November 2022 in Japan. Whereas, single-agent selumetinib has no evidence of efficacy in MPNST, and its efficacy in intermediate- tumor is uncertain. Appropriate use of selumetinib in symptomatic PN is important to ensure that the PN has not become malignant. Post-approval clinical use experience is critical data to evaluate the safety and efficacy of selumetinib, especially in Asian patients. It is also important to investigate whether selumetinib is used appropriately in non-malignant PN. The purpose of this study is to report the short to middle-term use experience after selumetinib was approved in Japan in November 2022.

Methods: Subjects were patients for whom prescription of selumetinib was started for symptomatic, inoperable plexiform neurofibromas in children with neurofibromatosis type 1 at our institution for 6 months from November 16, 2022 to May 31, 2023. Prescription of selumetinib was determined by appropriately evaluating the following items. (1) symptomatic, inoperable PN: “Symptomatic” was defined as causing ADL/QOL impairment in patients. “Inoperability” was determined by PN site, including orthopedic oncologist, head and neck surgeon, neurosurgeon, and plastic surgeon. Our facility has a multidisciplinary clinical team for NF1 patients Clinical factors of patients, site of symptomatic PN, maximum diameter, dose of selumetinib, administration period, adverse events (AE), presence or absence of dose reduction were investigated. (2) Evaluation of non-malignant transformation: Careful evaluation was performed using contrast-enhanced MRI, diffusion-weighted imaging, and FDG PET-CT, and if necessary, biopsy was performed to confirm intermediate type and no malignant transformation. Clinical factors of patients, site of symptomatic PN, maximum diameter, dose of selumetinib, administration period, adverse events (AE), presence or absence of discontinuation or dose reduction of selumetinib were investigated.

Results: Selumetinib was prescribed to a total of 11 patients, 7 males, mean age 14 years (8-18). The sites of symptomatic PN were the head in 5 patients, the pelvis to the lower extremities in 3 patients, and the paraspinal lesions in 3 patients. The morbidities of the patients were disfigurement in 6 patients, pain and motor dysfunction in 5 patients, and impending spinal cord injury in 1 patient. The median maximum diameter of PN was 91 mm (39-814). The dose of selumetinib was 50 mg/day in 4 patients, 60 mg/day in 4 patients, and 70 mg/day in 3 patient. The median treatment duration was 4 months (range: 1-6 months). Grade 3 or higher AE occurred in only 1 patient, which was elevated ALT. Grade 2 AEs were diarrhea in 4 cases, rash in 1 case, acne in 1 case, and pruritus in 1 case . There were 2 patients of temporary withdrawal of selumetinib due to AE. One is due to Grade 2 diarrhea, and the other is due to more than 10% decreased ejection fraction. Both patients plan to resume oral administration of selumetinib.

Conclusion: Six months have passed since selumetinib was prescribed for symptomatic inoperable PN in children with NF1 in Japan. Despite the short-term results, the prescription was continued in most patients without major AEs. It is necessary to create data on efficacy and safety through patient accumulation.

(P 164) INTRATUMORAL INT230-6 (CISPLATIN, VINBLASTINE, SHAO) ALONE OR WITH IPILIMUMAB PROLONGED SURVIVAL WITH FAVORABLE SAFETY AND IMMUNE ACTIVATION IN ADULTS WITH REFRACTORY SARCOMAS (NCT 03058289)

Christian F. Meyer, MD PhD[1]; Matthew A. Ingham, MD[2]; James S. Hu, MD[3]; Giles Whalen, MD[4]; Jacob Thomas, MD[3]; Anthony B. El-Khoueiry, MD[5]; Diana Hanna, MD[3]; Luis Camacho, MD MPH[6]; Anthony J. Olszanski, MD RPh[7]; Nilofer S. Azad, MD[8]; Franco Abbate, PhD[9]; Lewis H. Bender, M.S., M.A., M.B.A.[10]; Ian B. Walters, MD, MBA[9]; Lillian L. Siu, MD[11]; Albiruni Ryan Abdul Razak, MB BCh, BM BCh[12], (1) Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, (2) Columbia University Medical Center, New York, NY, (3) University of Southern California, Norris Comprehensive Cancer Center, Los Angeles, CA, (4) University of Massachusetts Memorial Medical Center, Worcester, MA, (5) University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA, (6) Center for Onc and Blood Disorders, Houston, TX, (7) Fox Chase Cancer Center, Philadelphia, PA, (8) Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Hospital, Baltimore, MD, (9) Intensity Therapeutics, Westport, CT, (10) Intensity Therapeutics Inc., S, CT, (11) Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada, (12) Princess Margaret Cancer Center, Toronto, ON, Canada

Objective: Sarcomas are a rare and diverse group of solid tumors from mesenchymal cells; chemotherapy provides limited benefit for metastatic disease. INT230-6 is a novel formulation of cisplatin (CIS), vinblastine (VIN) and a tissue dispersion enhancer (SHAO) designed for intratumoral (IT) delivery. The drug diffuses into cancer cells, causes apoptosis and recruits dendritic and T-cells to the tumor. Adding ipilimumab (IPI) appears to improve INT230-6 responses in models. The primary objective of this study was to understand the safety of INT230-6 monotherapy or in combination with IPI. Secondary objectives were to understand efficacy disease control rate (DCR) and overall survival (OS) of the drug alone or with intravenous (IV) IPI in various highly refractory sarcoma subtypes.

Methods: IT-01 is an open-label phase1/2 study in adults with locally advanced, unresectable or metastatic solid tumors, including sarcoma. INT230-6 dose was set by tumor diameter or volume. INT230-6 was dosed IT Q2W for up to 5 doses alone or with IPI at 3mg/kg Q3 weeks for 4 doses. Maintenance INT230-6 dosing was Q9 weeks. Immunohistochemistry was conducted on biopsied tissue from pre- and post-dosed tumors2.

Results: The study enrolled 30 sarcoma patients (15 INT230-6 alone, 14 combination) with 11 subtypes (primarily leiomyosarcoma, liposarcoma, pleomorphic, chondrosarcoma and chordoma) baseline demographics in Table 2. There were no dose limiting toxicities in treating sarcoma at doses up 175 mL (87.5 mg of CIS, 17.5 mg VIN) following injection into 1 or more tumors, amounts that approach or exceed an IV dose of CIS or VIN. PK analysis shows that 95% to 99.5% of VIN remained in the tumor. Cisplatin is rapidly degraded and only platinum metal is measured. Data showed that INT230-6 induced an immune response in sarcoma subtypes as a monotherapy with increased CD4+ T-cell influx. Of 29 evaluable patients for efficacy, the DCR was 93% for monotherapy and 86% combined with IPI. RECIST measurements are confounded by IT injections due to the large volume of highly retained INT230-6 and the influx of immune infiltrates; however, 27% of sarcoma subjects receiving only local INT230-6 uninjected tumors shrink (many nontarget tumors were unreported). Analysis of median overall survival (mOS) for INT230-6 alone (n=15) was 21.3 months. It was observed empirically during the study that subjects having a cumulative dose of INT230-6 at greater than 40% drug volume to total tumor burden (TTB) had extended survival. For monotherapy subjects

receiving a dose of ≥40% of TTB (n=11) the mOS had not been reached after 13.5 months of median follow-up . The mOS of the combination has also not been reached with over 12 months of median follow-up. The treatment-related adverse events (TRAEs) in evaluable monotherapy patients were mild; 13.3% grade1 and 60% grade 2. Grade 3 TRAEs occurred in 20% and 7% of patients in the monotherapy and combination arms respectively. There were no grade 4 or 5 events. The >20% Treatment Emergent Adverse Events Related to Drug Regimen in Evaluable Patients are shown in the below Table 1

Conclusion: INT230-6 use was associated with immune infiltration and a clinically relevant mOS particularly when ≥40% of the TTB was injected. Uninjected tumors shrank in a high percentage of monotherapy subjects. INT230-6 dosed IT alone or with IPI had favorable safety in diverse sarcomas. A randomized phase 3 trial protocol vs. SOC in selected sarcoma subtypes with an OS endpoint has been reviewed by the US FDA.

Table 1 Treatment Related Adverse Events

Event	INT2306 (n=15)	INT230-6 + IPI (n=14)
Localized pain	80%	39%
Nausea	40%	28%
Fatigue	33%	39%
Decreased appetite	27%	*
Vomiting	20%	21%
Pruritus	*	21%
Rash	*	21%

* <20%

(P 165) MIDDLE EAR RHABDOMYOSARCOMA: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP

Sarah Whittle, MD[1]; Wei Xue, PhD[2]; Suzanne Wolden, MD[3]; Carola Arndt, MD[4]; Douglas S. Hawkins, MD[5]; Rajkumar Venkatramani, MD[1], (1) Baylor College of Medicine, Houston, TX, (2) University of Florida, Gainsville, FL, (3) Memorial Sloan Kettering Cancer Center, New York, NY, (4) Mayo Clinic, Rochester, MN, (5) Seattle Children's Hospital, Seattle, WA

Objective: Middle ear rhabdomyosarcoma (ME RMS) is often locally advanced at diagnosis with extension occurring through the tympanic membrane, intracranially, or laterally in front of or under the ear, but rarely metastasizes distantly. Prior studies suggest ME RMS may have a more favorable outcome than other parameningeal sites. We describe the presentation and outcomes of patients with ME RMS treated on recent Children's Oncology Group clinical trials.

Methods: Patients with ME RMS enrolled on Children's Oncology Group Studies for newly diagnosed rhabdomyosarcoma from 1997 to 2013 were identified. Demographic information, tumor characteristics and survival information were analyzed using descriptive and survival statistics.

Results: Forty-four patients with ME RMS were identified. Twenty-seven (61.4%) were treated on D9803, 16 (36.4%) on ARST0531, and 1 (2.3%) on ARST0431. There were no patients with ME RMS treated on D9602, D9802, ARST0331, or ARST08P1. The majority (65.9%) were male, and between 1 to 9 years old (97.7%). Tumor histology was most commonly botryoid RMS (43.2%), followed by embryonal RMS (38.6%), with only 4.5% alveolar histology. The

majority (77.3%) had tumors less than or equal to 5 cm in greatest diameter. Lymph node involvement was identified in 22.7%, and distant metastatic disease in only 4.5%. Most tumors were classified as stage 2 (59.1%) or stage 3 (36.4%), with the vast majority being IRS Group III (93.2%). Most patients (97.7%) received radiation therapy. The 4-year event free survival (EFS) for the whole cohort was 75%% (95% CI 61.8-88.2), and overall survival (OS) 79.3% (95% CI 66.1-92.0). There was no difference in EFS or OS related to tumor size. While there was no significant difference between outcomes related to trial of entry, it is notable that the 4 year EFS and OS on D9803 were 81.55% (95% CI 66.8-96.1) and 85.2% (95% CI 71.5-98.9) compared with 62.5% (95% CI 35.9-89.0) and 67.7% (95% CI 41.0-94.4) on ARST0531. The single patient enrolled on ARST 0431 was a long-term survivor.

Conclusion: While not statistically significant, we demonstrate a trend toward worse EFS and OS for patients treated on ARST 0531, which used a lower cumulative cyclophosphamide dose than study D9803. This is consistent with a prior report demonstrating a significantly higher local failure rate with lower dose cyclophosphamide for head and neck rhabdomyosarcoma and a trend toward worsened EFS. (PMID 30508617)

(P 166) SARC038: A PHASE 2 STUDY OF REGORAFENIB IN COMBINATION WITH NIVOLUMAB IN PATIENTS WITH REFRACTORY OR RECURRENT OSTEOSARCOMA

Fariba Navid, MD[1]; Lara Davis, MD[2]; Scott M. Schuetze, MD, PhD[3]; Rachel Heise, MS[4]; Brian Turpin, DO[5]; Brenda Weigel, MD[6]; Kelly Klega, MS, MLS(ASCP)[7]; Brian Crompton, MD[7]; Shahab Asgharzadeh, MD[8]; Linda Cambier, PhD[8]; David Cobrinik, MD, PhD[8]; Andrew Y. Koh, MD[9]; Troy McEachron, PhD[10]; Karla V. Ballman, Ph.D.[4]; Robert G. Maki, MD PhD[11]; Leo Mascarenhas, MD, MS[12], (1) Children's Hospital Los Angeles, Los Angeles, CA, (2) OHSU Knight Cancer Institute, Portland, OR, (3) University of Michigan, Ann Arbor, MI, (4) Weil Cornell Graduate School of Medical Sciences, New York, NY, (5) Cincinnati Children's Hospital Medical Center, Cincinnati, OH, (6) Department of Pediatrics, University of Minnesota, Minneapolis, MN, (7) Dana-Farber Cancer Institute, Boston, MA, (8) Children's Hospital Los Angeles, Los Angeles, CA, (9) University of Texas Southwestern Medical Center, Dallas, TX, (10) National Institutes of Health, Bethesda, MD, (11) Memorial Sloan Kettering Cancer Center; Professor, Weill Cornell Medical College, New York, NY, (12) Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA

Objective: Combination VEGF targeted agents and checkpoint inhibitors have shown benefit in a variety of cancer types. Regorafenib, a multi-tyrosine kinase inhibitor, has shown benefit in patients with refractory/recurrent osteosarcoma in two randomized placebo-controlled trials, REGOBONE and SARC024. We hypothesized that the addition of nivolumab, a PD-1 inhibitor, to regorafenib will improve the progression-free survival (PFS) rate of patients with refractory/recurrent osteosarcoma compared to a similar patient population who received regorafenib alone.

Methods: SARC038 is a single-arm, historically-controlled phase 2 study utilizing a Simon's 2-stage design. Patients ≥5 years of age with recurrent or refractory osteosarcoma, RECIST measurable disease after at least one line of prior systemic therapy and no prior exposure to VEGF targeted agents or checkpoint inhibitors were eligible. Treatment included nivolumab 480 mg IV (3 mg/kg days 1 and 15, if < 18 years of age) on day 1 and regorafenib 80-120 mg PO (60-72 mg/m2/dose, if < 18 years of age) days 1-21 in 28-day cycles. The primary endpoint was

PFS rate at 4 months as determined by iRECIST. If 11 or more patients of the initial 20 patients were progression-free at 4 months, the trial would proceed to the second stage to enroll an additional 23 patients. The trial was designed to have a one-sided 0.1 level of significance and 80% power to detect a 4-month PFS rate of 0.65 compared to an historical control value of 0.48 (based on patients treated with regorafenib alone). Secondary endpoints included objective response rate (ORR) and PFS using RECIST and iRECIST, overall survival and adverse events.

Results: Between June 2021 and January 2023, 23 patients [12 female, median age of 22 (range, 9-73 years)] were enrolled. A median of 4 cycles (range, 1-25) of therapy were administered as of May 12, 2023. All patients were evaluable for toxicity and 20 were evaluable for the primary endpoint. Eight of 20 were progression free for at least 4 months, resulting in a 4-month PFS of 46.3% (95% CI: 28.3-75.8%), which did not meet criteria to proceed to stage 2. With a median follow up time of 5.4 months (range, 0.5-22.0), the median PFS was 3.6 months (95% CI: 1.8-NA). One-year survival was 55.1% (95% CI: 30.6-99.4%). Twenty patients were evaluable for ORR, one had a partial response. Eighteen treatment-related adverse events of grade ≥3 occurred in 12 (52.2%) of the patients, with rash (including hand-foot skin reaction) being the most common (N = 6). Thrombocytopenia and leucopenia were observed in 2 patients each and anemia, lymphopenia, elevated AST, back pain, diarrhea, oral mucositis and wound dehiscence were observed in one patient each. The severity and frequency of rash prompted an amendment to use an alternative dosing schedule of regorafenib, starting at a lower dose and escalating to higher dose if well tolerated for one week.

Conclusion: Overall, the combination of regorafenib and nivolumab was generally well tolerated; however, rash was a frequently observed adverse event requiring dose reduction of regorafenib. Although the study did not meet the protocol defined criteria for efficacy to proceed to the second stage, a subset of patients receiving the combination of nivolumab and regorafenib did benefit from the therapy. Analysis of clinical characteristics and biospecimens (blood, tumor tissue and stool) collected at baseline, during and at the time of progressive disease is ongoing to understand potential predictors of efficacy.

(P 167) FDG-PET-CT RESPONSE AFTER NEOADJUVANT CHEMOTHERAPY PREDICTS LONG-TERM OUTCOME IN PATIENTS WITH SOFT TISSUE SARCOMA -- 15-YEAR FOLLOW-UP

Andrea P. Espejo-Freire, MD[1]; Edward Cheng, MD[2]; Juan Carlos Manivel, MD[2]; Jerry W. Froelich, MD[3]; Brenda Weigel, MD[4]; Denis Clohisy, MD[2]; Christian Ogilvie, MD[5]; Bruce Lindgren, PhD[2]; Richard Evans, PhD, PSTAT[6]; L. Chinsoo Cho, MD, MS[1]; Kathryn Dusenbery, MD[2]; Keith SKubitz, MD[7], (1) University of Minnesota, MInneapolis, MN, (2) University of Minnesota, Minneapolis, MN, (3) University of Minnesota, Minnetonka, MN, (4) Department of Pediatrics, University of Minnesota, Minneapolis, MN, (5) University of Minnesota, Excelsior, MN, (6) Univesity of Minnesota, Minneapolis, MN, (7) University of Minnesota, Edina, MN

Objective: Only 30 to 40% of high-grade sarcomas are responsive to initial chemotherapy (CX). CX has significant toxicities, and the evaluation of response by CT imaging has well-known limitations. FDG PET-CT has the advantage of assessing tumor metabolism in addition to tumor size changes. This prospective study evaluated whether early SUV-max changes on FDG PET-CT after neoadjuvant CX could predict outcomes in patients with high-grade sarcomas.

Methods: This prospective trial was designed to determine if the change in the SUV-max on PET after 1 or 4 cycles of chemotherapy could predict progression-free survival (PFS), overall survival (OS), and histologic response in patients with high-grade soft-tissue sarcomas receiving neoadjuvant CX with doxorubicin (free or pegylated-liposomal doxorubicin) and ifosfamide. We calculated a sample size of 56 patients to achieve 90% power to test the hazard ratio using a one-sided log-rank test at alpha = 5%. PET metabolic response was defined as the SUV-max change from baseline to following either cycle 1 of CX (initial response/Delta-1) or cycle 4 of CX before surgical excision (multiple cycle response/Delta-2) on an intent-to-treat basis. A positive responder was defined as a reduction in SUV-max reduction of >40% from baseline. Results were also analyzed using SUV-max as a continuous variable.

Results: Table 1 shows demographic characteristics. Sixty-four patients had evaluable data for Delta-1, and 56 had received additional chemotherapy cycles and were thus evaluable for a Delta-2 assessment. The long-term follow-up data was updated in May 2021 with a median follow-up time of 8.1 years (Range 0.4 to 14.9)
The 5-year PFS for patients with positive Delta-1 response was 65% vs. 34% for non-responders (p 0.021). This remained true after all chemotherapy cycles had been completed. Five-year PFS for Delta-2 responders was 63% vs. 26% in non-responders (p=0.002). Among the group of non-metastatic disease patients, 5-year PFS for Delta-1 responders was 72% vs. 42% (p=0.023) in non-responders, and 5-year PFS for Delta-2 responders 65% vs. 38% in non-responders (p=0.045). After Cox regression analysis, we found that PET-CT response was independently highly predictive of PFS. This was true regardless of whether the response was considered a continuous or categorical variable.
Similarly, a decrease in SUV-max predicted OS and correlated with histological response. 5-yr OS in Delta-1 responders was 73% vs. 56% in non-responders (p 0.1), and in Delta-2 responders 88% vs. 30% in non-responders (p< 0.001). For histological response, the correlation with Delta-1 response was 0.29 (p=0.026), n=59, and for Delta-2, the correlation was 0.51 (p< 0.001), n=52.
Importantly, long-term adverse events were noted. Two patients developed acute renal failure requiring dialysis and were left with chronic kidney disease. Two patients developed myelodysplastic syndrome and died from the disease. Three patients developed secondary malignancy (1 breast cancer, 1 radiation-induced chondrosarcoma, and 1 melanoma), and 1 developed autoimmune hemolytic anemia (AIHA).

Conclusion: This study demonstrates that the change in PET-CT SUV-max significantly predicts long-term outcomes in patients with high-grade sarcomas undergoing neoadjuvant anthracycline-based CX. This data supports using PET-CT early in treatment to assess response to anthracycline-based CX in patients with high-grade soft tissue sarcomas. In practice, assessing the response to CX with early PET-CT adds information to the decision to continue chemotherapy which is often challenging.
Finally, in our long-term follow-up analysis, 5 out of 64 patients developed secondary malignancies. This suggests that secondary malignancies and other serious adverse events after perioperative CX may be higher than captured previously by studies with shorter follow-ups.

Variable	N	Mean (SD)	Median [Range]
Age in years	69	52.5 (13.5)	54.0 [20-79]
Variable	**N**	**Percent (%)**	
Gender			
Male	42	62.9	
Female	27	39.1	
AJCC			
3	44	63.8	
4	25	36.2	
Disease status at last follow up	**N**	**Percent (%)**	
Alive, no progression	34	49.3	
Alive, progression	13	18.8	
Dead of disease	22	31.9	
Histology	**N**	**Percent (%)**	
Undifferentiated sarcoma	42	61	
Synovial sarcoma	15	22	
Leiomyosarcoma	4	6	
Liposarcoma	4	6	
Fibrosarcoma	3	4	
MPNST	1	1	

TABLE 1. Baseline characteristics of the 69 patients with high-grade sarcoma who underwent neoadjuvant chemotherapy and planned to have an FDG-PET-CT at baseline and after cycles one and 4 of chemotherapy.

(P 168) A PHASE 3 STUDY OF THE EFFICACY AND SAFETY OF PEXIDARTINIB IN ASIAN PATIENTS WITH TENOSYNOVIAL GIANT CELL TUMOR

Hairong Xu, MD[1]; Po-Kuei Wu, MD, PhD[2]; Zhaoming Ye, N/A, MD[3]; Zhengfu Fan, MD[4]; Tao Li, M.D.[5]; Tom Wei-Wu W. Chen, MD, PhD[6]; Jingnan Shen, MD[7]; Wangjun Yan, MD[8]; Issei Achiwa, PhD[9]; Kazukoh Yoh, MD, PhD[10]; Kunika Kikumori, MS, PharmD[10]; Yoshiharu Hiruma, PhD[10]; Jonathan Greenberg, BA, MA, MD[11]; Xiaohui Niu, MD[12], (1) Department of Orthopaedic Oncology Surgery, Beijing Ji Shui Tan Hospital, Peking University, Beijing, Beijing, China (People's Republic), (2) Taipei Veterans General Hospital, Taipei, Taipei, Taiwan (Republic of China), (3) Department of Orthopedics, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, China (People's Republic), (4) Peking University Cancer Hospital, Beijing, Beijing, China (People's Republic), (5) Department of Bone and Soft tissue Surgery, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, China (People's Republic), (6) National Taiwan University Hospital, Taipei, Taipei, Taiwan (Republic of China), (7) Department of Musculoskeletal Oncology Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China (People's Republic), (8) Fudan University Shanghai Cancer Center, Shanghai, Shanghai, China (People's Republic), (9) Daiichi Sankyo, Co., Ltd, Funabachishi, Chiba, Japan, (10) Daiichi Sankyo, Co., Ltd., Tokyo, Tokyo, Japan, (11) Daiichi Sankyo, Inc., Basking Ridge, NJ, (12) Department of Orthopaedic Oncology Surgery, Beijing Ji Shui Tan Hospital, Beijing, Beijing, China (People's Republic)

Objective: Tenosynovial giant cell tumor (TGCT), a rare mesenchymal neoplasm of the synovium, bursae, or tendon sheath, is associated with overexpression of colony-stimulating factor 1 (CSF-1). Pexidartinib, a novel, orally administered, selective inhibitor of the CSF-1 receptor, is approved by the United States Food and Drug Administration and Korean and Taiwan authorities for the treatment of adult patients with symptomatic TGCT associated with severe morbidity or functional limitations and not amenable to improvement with surgery. This approval was based on the double-blind, randomized, placebo-controlled phase 3 ENLIVEN study (ClinicalTrials.gov identifier: NCT02371369) that demonstrated robust tumor response and improved symptoms and functional outcomes with pexidartinib compared to placebo in patients with symptomatic, locally advanced TGCT. The current study evaluated the efficacy and safety of pexidartinib in Asian patients with TGCT.

Methods: This multicenter, single-arm, phase 3 study in China and Taiwan (ClinicalTrials.gov identifier: NCT04488822) enrolled adult patients (≥18 years) with symptomatic TGCT associated with severe morbidity or functional limitation and not amenable to improvement with

surgery. Patients received pexidartinib 400 mg (2 x 200 mg capsules) twice daily on an empty stomach. Pexidartinib was administered continuously on a 28-day treatment cycle until criteria for discontinuation was reached. The primary efficacy endpoint was overall response rate (ORR) defined as complete response (CR) or partial response (PR) by RECIST v1.1 at Week 25 by centrally reviewed MRI scans. Secondary endpoints included centrally reviewed ORR by tumor volume score (TVS) at Week 25, mean (standard error [SE]) change from baseline to Week 25 in range of motion (ROM) of affected joints, best overall response (CR or PR) by RECIST v1.1 and treatment-emergent adverse events (TEAEs). Data cutoff for the primary analysis was October 27, 2021, after all patients completed Week 25.

Results: Overall, 40 patients received pexidartinib. All patients were Asian; mean (standard deviation [SD]) age was 40.9 (12.05) years and 27 (67.5%) were female. Tumor response at Week 25 by RECIST v1.1 and TVS is shown in the Table. Overall least squares mean (SE) change from baseline at Week 25 in ROM was 23.05 (3.207; 95% CI: 16.40, 29.69), with improvement noted in the affected joint at Week 13 (15.64 [3.312]; 95% CI: 8.84, 22.44). Best overall response by RECIST v1.1 was observed in 12 (30.0%) patients, including 2 (5.0%) with CR; 20 (50.0%) patients had stable disease, and 1 (2.5%) patient had progressive disease. All patients reported TEAEs and were related to pexidartinib. NCI-CTCAE grade ≥3 TEAEs were reported in 16 (40.0%) patients, with 12 (30.0%) patients reporting treatment-related grade ≥3 TEAEs. There were no grade 5 TEAEs. The most common TEAE was hair color changes (31 [77.5%]), followed by increases in alanine aminotransferase (24 [60.0%]), aspartate aminotransferase (23 [57.5%]), and blood lactate dehydrogenase (19 [47.5%]); pruritus (19 [47.5%]); and rash and face edema (each 17 [42.5%]). Sixteen (40.0%) patients had AST ≥3 × ULN or ALT ≥3 × ULN, 9 (22.5%) patients had AST ≥5 × ULN or ALT ≥5 × ULN. Most of the events occurred within the first 2 study treatment cycles. There were 2 (5.0%) patients who had AST or ALT ≥ 3 × ULN with total bilirubin ≥2 × ULN.

Conclusion: In Asian patients with symptomatic TGCT not amenable to improvement with surgery, pexidartinib demonstrated clinical benefit in tumor response and improvement in joint function. The overall safety profile of pexidartinib was comparable with previous data. Hepatotoxicity was manageable with frequent liver test monitoring and dose modifications as defined in the protocol, and no new safety signals were observed in this Asian population.

Table: Tumor response at Week 25 by RECIST v1.1 and TVS

Tumor response at Week 25, n (%; 95% CI)	All pexidartinib treated (N = 40)	
	By RECIST v1.1	By TVS
ORR (CR or PR)	9 (22.5; 10.8-38.5)	19 (47.5; 31.5-63.9)
CR	0 (0; 0-8.8)	0 (0; 0-8.8)
PR	9 (22.5; 10.8-38.5)	19 (47.5; 31.5-63.9)
Stable disease	21 (52.5; 36.1-68.5)	12 (30.0; 16.6-46.5)
Progressive disease	1 (2.5; 0.1-13.2)	0 (0; 0-8.8)
Not evaluable	9 (22.5; 10.8-38.5)	9 (22.5; 10.8-38.5)

CI, confidence interval; CR, complete response; ORR, overall response rate; PR, partial response; TVS, tumor volume score.

(P 169) UPDATED EFFICACY AND SAFETY PROFILE OF PIMICOTINIB (ABSK021) IN TENOSYNOVIAL GIANT CELL TUMOR (TGCT): 1-YEAR FOLLOW-UP FROM PHASE 1B

Hairong Xu, MD[1]; Yong Zhou, N/A, M.D.[2]; Binghao Li, N/A, M.D.[3]; Xianbiao Xie, N/A, M.D.[4]; Xinhui Du, N/A, M.D.[5]; Bo Chen, M.D.[6]; Ting Yuan, M.D.[7]; Qingping Zou, N/A, PhD[8]; Qiuxiang Guo, N/A, n/a[9]; Boyao Shan, N/A, n/a[9]; Yang Dong, N/A, M.D.[7]; Zhichao Tong, M.D.[6]; Weitao Yao, MD[5]; Jingnan Shen, MD[4]; Zhaoming Ye, N/A, MD[3]; Chongqi Tu, M.D.[2]; Xiaohui Niu, MD[10], (1) Department of Orthopaedic Oncology Surgery, Beijing Ji Shui Tan Hospital, Peking University, Beijing, Beijing, China (People's Republic), (2) Department of Orthopedics, West China Hospital, Sichuan University, Chengdu, Sichuan, China (People's Republic), (3) Department of Orthopedics, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, China (People's Republic), (4) Department of Musculoskeletal Oncology Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China (People's Republic), (5) Department of Orthopedics, The Affiliated Cancer Hospital of Zhengzhou University and Henan Cancer Hospital, Zhengzhou, Henan, China (People's Republic), (6) Department of Osteopathic Oncology, Honghui Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi, China (People's Republic), (7) Department of Orthopedics, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, Shanghai, China (People's Republic), (8) Abbisko Therapeutics Co., Ltd., Shanghai, Shanghai, China (People's Republic), (9) Abbisko Therapeutics Co., Ltd, Shanghai, Shanghai, China (People's Republic), (10) Department of Orthopaedic Oncology Surgery, Beijing Ji Shui Tan Hospital, Beijing, Beijing, China (People's Republic)

Objective: TGCT is a rare class of locally aggressive neoplasm, predominantly driven by overexpression of colony-stimulating factor 1 (CSF-1) gene. Pimicotinib, an orally administered

selective small-molecule antagonist of CSF-1R, exhibits minimal inhibition of c-Kit and PDGFR. In 2023, the U.S. FDA conferred Breakthrough Therapy Designation and the European Medicines Agency granted Priority Medicine Designation upon Pimicotinib treatment of TGCT. Here, we report the phase 1b safety and efficacy results of Pimicotinib in TGCT patients (pts) over a 1-year follow-up.

Methods: The study (NCT04192344) entailed a TGCT cohort to evaluate the safety and preliminary antitumor activity of Pimicotinib in TGCT pts not amenable to surgical resection. The study investigated two dosage regimens: 50 mg once daily (QD) and 25 mg QD. As of 31 May 2023, a total of 56 TGCT pts were enrolled, comprising 44 pts in the 50 mg QD and 12 pts in the 25 mg QD cohort.

Results: Median age of 56 TGCT pts was 39 y (range: 18-76) and 41.1% were male. Tumors were primarily located in knee (48.2%), hip (16.1%) or ankle (10.7%). Prior to enrollment, thirty-three pts (58.9%) had undergone at least one tumor resection surgery. Extended follow-up indicated that Pimicotinib was well-tolerated, with a median treatment duration of 12.2 mos and the maximum treatment duration being 17.5 mos. 83.9% pts remained on treatment. A sustained improvement has been observed relative to the 6-month data earlier reported in CTOS last year, with an ORR of 87.5% (28/32, including 3 CR, 95%CI: 71.01%-96.49%) in 50 mg QD cohort and 66.7% (8/12, including 2 CR, 95%CI: 34.89%-90.08%) in 25 mg QD cohort by IRC based on RECIST 1.1 in response-evaluable analysis set. Out of the 16 pts in 50 mg QD cohort who achieved a PR within the first 6 mos and had follow-up data available, 15 of them (93.8%) sustained this response beyond 6 mos. Out of 5 pts in 50 mg QD cohort who achieved SD within the first 6 mos and had follow-up data, 4 (80%) pts improved to PR after 6 mos. Median DOR was not reached in either cohort. Durable improvements in range of motion, stiffness and pain over a 1-year follow-up were observed in both dose cohorts. The majority of TEAEs were Grade 1 to 2. Most common drug-related TEAEs (≥15%) include LDH increase (80.4%), CPK increase (67.9%), α-HBDH increase (62.5%), AST increase (42.9%), amylase increase (30.4%), ALT increase (25.0%), pruritus (21.4%), rash (19.6%), face oedema (19.6%) and dyslipidaemia (19.6%). No hair color change or serious liver injuries were reported, meanwhile, blood enzymes elevations were asymptomatic, within the expected range, and quickly recovered following drug interruptions, all of which were consistent with previous observations. Compared to the data within 6 mos, the overall safety profile remains largely consistent with no distinct adverse events emerging upon extended follow-up.

Conclusion: With the extension of treatment duration, there is an observed augmentation in the number of pts experiencing sustained tumor shrinkage and favorable safety of Pimicotinib with no apparent hepatotoxicity. Current data confers durable therapeutic benefits in TGCT pts, suggesting that prolonged exposure may represent an optimal treatment approach. In addition, a separate cohort with prior anti-CSF-1/CSF-1R therapies is underway to assess the safety and antitumor activity.

(P 170) HETEROGENEITY OF PAZOPANIB ACTIVITY ACROSS SOFT TISSUE SARCOMA SUBTYPES - A RETROSPECTIVE ANALYSIS OF 303 CASES FROM A REFERENCE CENTER

Pawel Sobczuk, MD PhD[1]; Anna Janik, MD[2]; Anna Jurga, n/a[2]; Anna Ziobro, n/a[2]; Hanna Koseła-Paterczyk, MD PhD[3]; Katarzyna Kozak, MD, PhD[4]; Tomasz Switaj, MD, PhD[2]; Pawel Rogala, MD PhD[2]; Slawomir Falkowski, MD PhD[2]; Piotr Rutkowski, MD, PhD[2], (1) Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (2) Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Mazowieckie, Poland, (3) Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (4) Maria Sklodowska-Curie National Research Institute of Oncology Warsaw Poland, Warsaw, Mazowieckie, Poland

Objective: Pazopanib was authorized for the treatment of soft tissue sarcoma (STS) over 10 years ago, and since then, any novel tyrosine kinase inhibitor has been approved in this indication. Median progression-free survival (PFS) achieved in the phase III PALETTE trial was modest (4.6 months), but pazopanib showed a nearly 70% reduction in risk of disease progression compared to the placebo. In the current study, we aimed to analyze real-world evidence of the activity of pazopanib in patients with advanced STS treated in a reference center.

Methods: We retrospectively analyzed the medical data of patients who commenced treatment with pazopanib between November 2012 and December 2021. Demographic and clinicopathological data were extracted. PFS, overall survival (OS), and duration of response (DoR) were estimated with the Kaplan-Meier method, and a comparison between groups was performed with a long-rank test. Cox regression was used for multivariate analysis. The growth Modulation index (GMI) was calculated as PFS on pazopanib divided by PFS on therapy given immediately before pazopanib. Metastases-free interval (MFI) was defined as the time from curative treatment to the development of metastatic or unresectable disease.

Results: A total number of 303 patients has been identified. The median age was 53 years (range 18-88), 50.2% (n=152) were female, 82.2% (n=249) underwent curative surgery for the primary tumor, and 24.8% (n=75) had distant metastases at diagnosis. The most common sarcoma subtypes were leiomyosarcoma (LMS; 28.4%; n=86), synovial sarcoma (SS; 18.2%, n=55), and undifferentiated pleomorphic sarcoma (UPS; 15.8%, n=48). The majority of patients received treatment at the metastatic stage (92.7%; n=281) in 3rd or 4th line of therapy, in 32.0% (n=97) and 31.7% (n=96), respectively. Lung (73.9%, n=224) was the most common location of metastases. 39.3% (n=119) of patients required a temporary hold of pazopanib, and 24.8% (n=75) required dose reduction. Median PFS was 3.7 months (95%CI 2.9-4.4). 44.2% (n=134) of patients had GMI>1.3, indicating a better activity than previous line therapy. There were significant differences in PFS depending on histological subtype, with the longest median PFS in solitary fibrous tumor (SFT) (11.1; 95%CI 9.2-12.9), desmoplastic small round cell tumor (DSRCT) (7.4; 95%CI 2.1-12.7) and SS (5.5; 95%CI 3.1-7.8). PFS was significantly longer in women (4.6 vs. 2.8; p< 0.001) and in patients with MFI>10 months (4.2 vs. 3.0; p=0.012). Multivariate analyses confirmed female gender, SFT and SS, and MFI>10 to be associated with improved PFS. Median OS was 8.3 months (95%CI 7.0-9.7) and was significantly longer in females than males (10.7 vs. 6.7; p< 0.001), patients receiving subsequent therapy (12.8 vs. 4.9; p< 0.001) and with MFI>10 (9.7 vs. 6.7; p< 0.001). Patients with SFT had the longest OS (17.4; 95%CI 8.9-26.0), followed by LMS (10.4; 95%CI 8.2-12.7). Multivariate analyses confirm female gender, SFT, and MFI>10 to be associated with improved OS. Patients who required

dose interruption or dose reduction had significantly longer PFS (5.9 vs. 2.7 months; p< 0.001) and OS (11.7 vs. 6.5; p< 0.001), but this could be associated with longer exposition to the drug.

Conclusion: In our cohort, pazopanib showed modest activity in the majority of STS with a median PFS shorter than in the pivotal trial. SFT showed exceptionally long PFS, longer than in dedicated clinical trials. Moreover, synovial sarcoma and DSRCT seem to be most sensitive to pazopanib. Besides histology, gender and MFI are the only factors associated with PFS and OS. Importantly, pazopanib is associated with significant toxicities and a large proportion of patients required dose adjustments or interruptions. Our results highlighted the need for new therapies in STS.

(P 171) SEX DIFFERENCES IN OUTCOMES AND TOXICITY IN PATIENTS TREATED FOR SARCOMA IN A REFERENCE CENTER

Ilaria Tortorelli, MD[1]; Benedetta Chiusole, MD[1]; Fabio Murtas, MD[1]; Antonella Galiano, MD[1]; Maital Bolshinsky, MD[1]; Selma Ahcene Djaballah, MD[2]; Chiara De Toni, n/a[1]; Salvatore Vizzaccaro[1]; Marco Maruzzo, MD, PhD[1]; Umberto Basso, MD[1]; Alberto Banzato, MD[3]; Marina Coppola, n/a[4]; Antonella Brunello, MD, PhD[5], (1) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Veneto, Italy, (2) Oncology 3 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Castelfranco Veneto, Veneto, Italy, (3) Cardiology Unit, Veneto Institute of Oncology IOV - IRCCS, Padua, Veneto, Italy, (4) Pharmacy Unit, Veneto Institute of Oncology IOV - IRCCS, Padua, Veneto, Italy, (5) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy, Padua, Veneto, Italy

Objective: In the era of precision medicine, despite mounting evidence that women might be more susceptible to toxicity from chemotherapy (CT), sex influence on treatment effects and the biology of non-sex-related cancers have largely been ignored. Due to their rarity, data on patients (pts) with sarcoma are scarce.

Methods: We reviewed data of pts with sarcoma treated with (neo)adjuvant and/or first line CT between 2015 and 2022 at Veneto Institute of Oncology. Delays/reductions were evaluated in the first 9 weeks of CT and expressed as relative dose intensity (RDI): ratio of delivered dose intensity (mg/m2 per week) to theorical dose intensity. Survival was analyzed by Kaplan-Meier, and comparisons among groups studied through log-rank and Cox regressions. Sex differences in RDI, adverse events (AEs) and impact of RDI on disease-free survival (DFS), progression-free survival (PFS) and overall survival (OS) were analyzed.

Results: Overall, data for 197 pts were available (109 treatments for localized disease: 69 neoadjuvant/40 adjuvant setting; 127 first line treatments for advanced disease). Median age was 57 years (IQR: 41-67; range: 17-83), males were 112 (57%). Soft tissue sarcomas were 167 (85%), bone sarcomas were 30 (15%). Most frequent histological subtypes were leyomiosarcoma, liposarcoma, angiosarcoma, Ewing-sarcoma, synovial sarcoma, undifferentiated pleomorphic sarcoma. Grade ≥ 3 AEs were observed in 60% of pts. Male had a lower risk of severe non-hematologic AEs (OR 0.5; p=0.04), while no significant sex differences were observed for all severe AEs. Dose was reduced upfront in 46% of female Vs 28% of males (p=0.007) . Also, males less often had RDI < 85% (OR 2.4; p=0.002). Survival outcomes were similar in both sexes, though comparisons might suffer from biases due to different distribution of histotypes within sexes. In the localized disease setting, worse survival was

observed for males receiving RDI < 85% compared to ≥85% (median OS 36.6 months, 95% CI 23.7-NA Vs 63.7 months, 95% CI 51.1-NA, p=0.004; median DFS 11.1 months, 95% CI 5.3-NA Vs 26.3 months, 95% CI 15.9-NA, p=0.03) whereas no significant differences at the 85% threshold of RDI were found for females.

Conclusion: Greater risk of AEs and lower RDI in women treated for sarcoma add to the evidence that a sex-difference in treatment delivery and outcomes exists. Despite a global lower RDI, survival outcomes for female were not worse compared to male. Interestingly, RDI < 85% did not negatively affect survival outcomes in women (standard dosing based on “male” standards?). Such data call for optimization of drug dosing by sex to be considered in trial design.

(P 172) PHASE 2 RESULTS FROM THE RINGSIDE PHASE 2/3 TRIAL OF AL102 FOR TREATMENT OF DESMOID TUMORS

Robin L. Jones, BSc, MB BS, MRCP, MD[1]; Mrinal Gounder, MD[2]; Rashmi Chugh, MD[3]; Mark Agulnik, MD[4]; Arun Singh, MD[5]; Brian Andrew Van Tine, MD, PhD[6]; Vladimir Andelkovic, MD[7]; Edwin Choy, MD, PhD[8]; Jeremy Lewin, MB BS[9]; Ravin Ratan, MD[10]; Howard A. Ball, PhD[11]; Jonathan Y. Yovell, MD[12]; Gary Gordon, MD, PhD[13]; Andres A. Gutierrez, MD, PhD[14]; Bernd Kasper, MD, PhD[15], (1) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (2) Memorial Sloan Kettering Cancer Center, New York, NY, (3) University of Michigan Rogel Cancer Center, Ann Arbor, MI, (4) City of Hope, Department of Medical Oncology & Therapeutics Research, Duarte, CA, (5) University of California Los Angeles, Santa monica, CA, (6) Washington University School of Medicine, St. Louis, MO, (7) Princess Alexandra Hospital and Icon Cancer Centre, South Brisbane, Queensland, Australia, (8) Mass General Cancer Center, Boston, MA, (9) Peter MacCallum Cancer Centre, Victoria, Victoria, Australia, (10) The University of Texas MD Anderson Cancer Center, Houston, TX, (11) Ball Pharma Consulting Group, Hartland, WI, (12) Ayala Pharmaceuticals, Rehovot, Tel Aviv, Israel, (13) Ayala Pharmaceuticals, Northbrook, IL, (14) Ayala Pharmaceuticals, Monmouth Junction, NJ, (15) University of Heidelberg, Mannheim University Medical Center, Mannheim, Baden-Wurttemberg, Germany

Objective: There is an unmet need for more effective and well tolerated systemic therapy for desmoid tumors (DT, aggressive fibromatosis). Gamma secretase inhibitors (GSIs) have shown antitumor activity against DT. AL102 is a potent, oral, selective GSI under investigation for treatment of DT.

Methods: RINGSIDE is a Phase 2/3 study for patients with progressing DT. In the open-label Phase 2 study, adults with progressing DT (≥10% unidimensional growth ≤18 months or DT-related pain requiring non-opioid medication) were randomized to 3 dose regimens: 1.2 mg QD (once daily), 2 mg intermittent BIW (2 days on 5 days off), or 4 mg intermittent BIW. RINGSIDE Phase 3 is a double-blind, placebo-controlled study evaluating the chosen dose regimen from Phase 2 (1.2 mg QD). We report updated efficacy and safety results from RINGSIDE Phase 2.

Results: All 42 Phase 2 patients enrolled as of Mar 2022. As of Jan. 3, 2023, median time on study was 10.5 months (range 0.8 – 14.7) and 30 patients (71%) were still on study, 10 (24%) of whom rolled over to an open-label extension. Mean age was 40 years, 74% were women, and 69% had received prior DT therapy. Pharmacokinetics data showed that AL102 was rapidly

absorbed at all doses and exposure was similar under fasted and fed conditions. The median tmax was 1-2 hours, the effective t1/2 was 19.4-22.4 hours and time to steady state after daily dosing was 4-5 days. The best response in the evaluable population by blinded independent central review (BICR) was partial response in 6/12 patients (50%) for 1.2 mg QD, 3/13 patients (23%) for 4 mg BIW, and 5/11 patients (46%) for 2 mg BIW. Disease control rate was 100%, 91%, and 97%, respectively. Deeper, more rapid and maintained response was observed with 1.2 mg QD. Median volume change (BICR) from baseline was -52% for 1.2 mg QD, -10% for 4 mg BIW, and -15% for 2 mg BIW at Week 16 and -76%, -36%, and -52%, respectively, at Week 28, with similar patterns for T2 signal intensity (reduced tumor cellularity). The 5 most common Grade 1-2 treatment-emergent adverse events (TEAE) were diarrhea, nausea, fatigue, alopecia, and dry skin. Grade 3 drug-related TEAE were reported in 10/42 (24%) patients. There were no Grade 4-5 or serious drug-related TEAE and no new safety signals. Final Phase 2 data will be reported.

Conclusion: In this Phase 2 study, AL102 was safe and well tolerated across all tested doses. The safety profile was consistent with the GSI class of drugs. Tumor response, volume reduction and T2 signal reduction were observed earlier in the 1.2 mg QD group, with deeper and maintained treatment responses. This dose was selected for study in RINGSIDE Phase 3, which is currently enrolling in multiple countries.

(P 173) SAFETY, EFFICACY, AND PATIENT-REPORTED OUTCOMES WITH VIMSELTINIB IN PATIENTS WITH TENOSYNOVIAL GIANT CELL TUMOR WHO RECEIVED PRIOR ANTI–COLONY-STIMULATING FACTOR 1 THERAPY: ONGOING PHASE 2 UPDATE

Andrew J. Wagner, MD, PhD[1]; Kristen N. Ganjoo, MD[2]; Gina Z. D'Amato, MD[3]; Silvia Stacchiotti, MD[4]; Hans Gelderblom, MD, PhD[5]; Christopher W. Ryan, MD[6]; Emanuela Palmerini, MD, PhD[7]; Vinod Ravi, MD, MS, MBA[8]; Ramy Saleh, MD, MSc, FRCPC[9]; Axel Le Cesne, MD[10]; Nicholas Bernthal, MD[11]; Michiel van de Sande, MD, PhD[5]; Supraja Narasimhan, MS, PhD[12]; Brooke S. Harrow, PhD[13]; Maitreyi G. Sharma, MD[12]; Rodrigo Ruiz-Soto, MD/MSc[12]; Matthew L. Sherman, MD[12]; William D. Tap, MD[14], (1) Dana-Farber Cancer Institute, Boston, MA, (2) Stanford Cancer Center, Palo Alto, CA, (3) Sylvester Comprehensive Cancer Center, Miami, FL, (4) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (5) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (6) OHSU Knight Cancer Institute, South Waterfront, Portland, OR, (7) IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (8) The University of Texas MD Anderson Cancer Center, Houston, TX, (9) McGill University Health Centre Research Institute, Montreal, PQ, Canada, (10) Gustave Roussy Institute, Villejuif, Ile-de-France, France, (11) University of California Los Angeles, Los Angeles, CA, (12) Deciphera Pharmaceuticals, LLC, Waltham, MA, (13) Deciphera Pharmaceuticals, LLC, WALTHAM, MA, (14) Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Tenosynovial giant cell tumor (TGCT) is a rare, locally aggressive neoplasm caused by upregulation of the colony-stimulating factor 1 (CSF1) gene, resulting in aberrant CSF1 expression and recruitment of CSF1 receptor (CSF1R)-dependent inflammatory macrophages. Vimseltinib is an investigational, oral switch-control tyrosine kinase inhibitor specifically designed to selectively and potently inhibit CSF1R. Here, we report updated safety, efficacy,

and patient-reported outcome (PRO) data from Cohort B of the phase 2 part (expansion) of an ongoing phase 1/2 study (NCT03069469) for patients (pts) with TGCT treated with vimseltinib who received prior anti-CSF1/CSF1R therapy.

Methods: Pts with TGCT not amenable to surgery who received prior anti-CSF1/CSF1R therapy (including pexidartinib, cabiralizumab, or vimseltinib) were enrolled and treated with vimseltinib 30 mg twice weekly (recommended phase 2 dose). The primary objective was to evaluate the safety and tolerability of vimseltinib. Exploratory objectives for Cohort B included assessing antitumor activity (by independent radiological review [IRR] using Response Evaluation Criteria in Solid Tumors version 1.1 [RECIST v1.1]) and PROs (reported for the 25-week timepoint). Pain was assessed by 2 items from the brief pain inventory (BPI; worst pain and average pain [baseline: average from a 2-week period during screening; week 25: average from weeks 2 and 3 of month 6]) and compared with tumor responses at 25 weeks (by IRR using RECIST v1.1).

Results: As of Jan 27, 2023, 15 pts with TGCT who received prior anti-CSF1/CSF1R therapy were enrolled (enrollment ongoing). Most pts (80%; 12/15) previously received pexidartinib and discontinued due to progression (n=3), toxicity (n=2), and other (n=7). Median age was 50 years (range, 26–65), and the most common tumor location was the knee (47%; 7/15). The safety profile remained consistent with prior reports. Most nonlaboratory treatment-emergent adverse events (TEAEs) were grade 1/2; grade 3/4 TEAEs in ≥5% of pts were elevated creatine phosphokinase, myalgia, fatigue, arthralgia, eczema, and pain in extremity. There was no evidence of cholestatic hepatotoxicity. One new treatment-related serious adverse event of squamous cell carcinoma of the skin was reported. TEAEs led to dose reductions and interruptions in 27% (4/15) and 60% (9/15) of pts, respectively. Median treatment duration was 7.6 months (range, 0.6–22.1; mean, 10.1 months); 67% (10/15) of pts were still on treatment at the time of this analysis. Reasons for treatment discontinuation included adverse events (n=2), physician decision (n=1), withdrawal by pt (n=1), and other (n=1). No pts progressed as assessed by IRR using RECIST v1.1 (median follow-up from first dose, 8.3 months). The objective response rate (ORR) was 58% (7/12; Table 1). The majority of responses (86%; 6/7) were achieved within 6 months after treatment initiation with a median time to first response of 3.7 months (range, 1.6–8.3). At week 25, 54% (7/13) of pts demonstrated ≥30% reduction in both BPI worst pain and average pain. All pts who had a tumor response at week 25 experienced ≥30% reductions in BPI worst pain and average pain (among responders with BPI data available at week 25; Table 2). Most pts with stable disease (SD) at week 25 also experienced ≥30% reductions in BPI worst pain and average pain.

Conclusion: Longer follow-up demonstrated that vimseltinib continued to be well tolerated with a manageable safety profile in pts with TGCT not amenable to surgery who received prior anti-CSF1/CSF1R therapy. Antitumor activity continued to improve in this pretreated population, with an increased ORR. At week 25, all responders experienced ≥30% reductions in worst and average pain. Pain reductions were also observed in pts with SD, suggesting that vimseltinib provides pain relief with or without an objective tumor response. Taken together, these data demonstrate that vimseltinib can provide antitumor activity and pain relief in pts with TGCT.

Table 1. Response as assessed by IRR using RECIST v1.1 in patients with TGCT receiving vimseltinib

	Patients with prior anti-CSF1/CSF1R therapy
Safety population, n	15
Efficacy-evaluable population, n	12
Best overall response, n (%)[a]	
Objective response rate	7 (58)
Complete response	0[c]
Partial response	7 (58)
Stable disease	5 (42)
Duration of response, median[b] (min, max), months	NR (3.0+, 18.0+)

+ indicates patients are ongoing on study.
[a]Best overall response by IRR using RECIST v1.1; includes all available follow-up.
[b]Based on Kaplan-Meier estimate. Duration of response is defined as time from first imaging results showing response to progressive disease.
[c]A previous complete response (from ESMO 2022) was changed to a partial response by IRR based on additional follow-up assessments.
CSF1, colony-stimulating factor 1; CSF1R, CSF1 receptor; IRR, independent radiological review; max, maximum; min, minimum; NR, not reached; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; TGCT, tenosynovial giant cell tumor.

Table 2. BPI worst pain and average pain correlated with tumor response at week 25 in patients with TGCT receiving vimseltinib

≥30% pain reduction at week 25, n (%)	Tumor response at week 25[a]	
	Partial response n = 3	Stable disease n = 4
Worst pain	3 (100)	3 (75)
Average pain	3 (100)	3 (75)

Includes patients with both BPI and tumor response data available at week 25 (n = 7); percentages represent the proportions of patients with partial response or stable disease with ≥30% pain reduction.
[a]By IRR using RECIST v1.1.
BPI, Brief Pain Inventory; IRR, independent radiological review; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; TGCT, tenosynovial giant cell tumor.

(P 174) SELISARC: A SPANISH GROUP FOR RESEARCH IN SARCOMA (GEIS) PHASE I/II TRIAL OF SELINEXOR PLUS GEMCITABINE IN SELECTED SARCOMA SUBTYPES: RESULTS OF THE PHASE I PART

Javier Martin-Broto, MD, PhD[1]; Antonio Casado, MD[2]; David S. Moura, PhD[3]; Luis Ortega, MD[4]; Andres Redondo, MD, PhD[5]; Daniel Bernabeu, MD[5]; Javier Martinez-Trufero, MD[6]; Claudia Valverde, MD[7]; Antonio Gutierrez, MD, PhD[8]; Jose L. Mondaza-Hernandez, Master degree[3]; Arancha Manzano, MD, PhD[9]; Gloria Marquina, MD, PhD[4]; Nadia Hindi, MD, MSc[1], (1) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain, (2) Hospital Universitario Clínico San Carlos, Madrid, Madrid, Spain, (3) Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM),, Madrid, Madrid, Spain, (4) Hospital Clínico San Carlos, Madrid, Madrid, Spain, (5) HU La Paz, Madrid, Madrid, Spain, (6) HU Miguel Servet, Zaragoza, Aragon, Spain, (7) Vall D'Hebron University Hospital, Barcelona, Catalonia, Spain, (8) HU Son Espases, Palma, Islas Baleares, Spain, (9) HU Clínico San Carlos, Madrid, Madrid, Spain

Objective: Selinexor (KPT-330) (S), a small-molecule inhibitor of the nuclear export mediator exportin-1 (XPO-1), is able to reduce mRNA and protein expression of DNA damage repair (DDR) gene products, being synergistic with DNA damage agents as gemcitabine (G) in preclinical experiments. We hypothesized that selinexor would have a synergistic effect with gemcitabine in patients with selected sarcoma subtypes.

Methods: Adult patients, with progressing sarcoma (based in RECIST), ECOG 0-1, with up to 2 previous systemic therapies for advanced disease (localized unresectable/metastatic) with centrally confirmed diagnosis of undifferentiated pleomorphic sarcoma (UPS), leiomyosarcoma (LMS), alveolar soft part sarcoma (ASPS), or osteosarcoma (OS) were eligible. Selinexor (days 1, 8, 15) and Gemcitabine (days 1, 8) were given in four dose levels, L1: S 60 mg + G 1,000 mg/m2 in 30 min, L2: S 60 mg + G 1,000 mg/m2 (10 mg/m2/min), L3: S 60 mg + G 1,200 mg/m2 (10 mg/m2/min), and L4: S 80 mg + G 1,200 mg/m2 (10 mg/m2/min). A -1 level was defined with S 60 mg + G 800 mg/m2 (30 min). A classic 3+3 design was used to determine the recommended phase 2 dose (RP2D) based on Dose-limiting toxicities (DLTs) observed during the first 21-day cycle. In vitro research was performed in LMS and OS cell lines to test the synergy of the combination (MTS and flow cytometry assays).

Results: Between November 2020 and June 2022, 17 patients (Male/Female 9/8), ECOG 0/1 (14/3), median age 50 years (22-71) were recruited. The median of previous lines was 1 (1-2). Diagnosis was: 9 LMS (52.9%), 6 OS (35.3%), 1 ASPS and 1 synovial sarcoma (SS) (5.9%). Three patients were treated in each of the first 3 levels and 8 patients in L4 (2 patients in that cohort were not evaluable for DLT due to improper dosing). Only one DLT was observed in L4 (G4 thrombocytopenia) and this level was selected as RP2D. G3/4 toxicity: Neutropenia (52.9%), thrombocytopenia (41.2%), febrile neutropenia (11.8%), anemia, nausea, asthenia, vomiting, alopecia, and lipase increased (5.9% each). There were 3 RECIST partial responses (PR), 7 stable disease (SD), and 7 progressive disease (PD). Median progression-free survival (PFS) for LMS was 7 months (95% CI: 3-11). No relevant clinical activity was observed in OS. In vitro cell viability studies shown that S+G was synergistic in the majority of LMS cell lines tested (combination index of 0.789 for CP0024, 0.791 for SK-UT-1, 0.604 for AA and 1.186 for IEC005). However, our results indicated antagonism of S+G in OS cell lines, with values for the combination index of 1.67 for MG63, 1.54 for SAOS2 and 1.23 for U2OS. Apoptosis assays by flow cytometry confirmed these observations in LMS and OS cells.

Conclusion: At the RP2D, S 80 mg + G 1,200 mg/m2 (10 mg/m2/min) is a feasible scheme with manageable toxicity. S+G has shown promising clinical activity for LMS, which warrants further investigation in a phase II. Preclinical studies shown the synergy and the antagonism of S+G in LMS and OS, respectively. Currently, a single-arm, Phase II study is currently being implemented.

(P 175) TRABECTEDIN PLUS RADIOTHERAPY ON SYMPHTOMATIC ADVANCED SARCOMA PATIENTS, RESULTS FROM THE SYNERGIAS STUDY: A SPANISH GROUP FOR RESEARCH IN SARCOMA (GEIS) PHASE II STUDY

Javier Martin-Broto, MD, PhD[1]; Katarina Majercakova, MD[2]; Ignacio Azinovic, MD[3]; Josefina Cruz, MD[4]; Laura Guzmán-Gómez, MD[5]; Rosa Álvarez Álvarez, PhD, MD[6]; Antonio Casado, MD[7]; Claudia Valverde, MD[8]; Andres Redondo, MD, PhD[9]; Raul Teres, MD[2]; Ana Alvarez, MD[10]; Maria Carmen Dolado, MD[11]; Anxela Doval, MD[12]; Belén Belinchon, MD[9]; Jose Merino, MD[3]; Ramiro Mendez, MD, PhD[13]; Patricio Ledesma, .[14]; Antonio Gutierrez, MD, PhD[15]; David S. Moura, PhD[16]; Nadia Hindi, MD, MSc[1], (1) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain, (2) Hospital de la Santa Creu i Sant Pau, Barcelona, Catalonia, Spain, (3) HU Fundación Jimenez Díaz, Madrid, Madrid, Spain, (4) HU Canarias, Santa Cruz de Tenerife, Canarias, Spain, (5) HU Fundación Jiménez Díaz, Madrid, Madrid, Spain, (6) Medical Oncology Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain., Madrid, Madrid, Spain, (7) Hospital Universitario Clínico San Carlos, Madrid, Madrid, Spain, (8) Vall D'Hebron University Hospital, Barcelona, Catalonia, Spain, (9) HU La Paz, Madrid, Madrid, Spain, (10) HU Gregorio Marañon, Madrid, Madrid, Spain, (11) HU Canarias, Tenerife, Canarias, Spain, (12) HU Clínico San Carlos, Madrid, Madrid, Spain, (13) Hospital Clinico san Carlos, Madrid, Madrid, Spain, (14) Sofpromed, Palma de Mallorca, Islas Baleares, Spain, (15) HU Son Espases, Palma, Islas Baleares, Spain, (16) Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM),, Madrid, Madrid, Spain

Objective: Pain relief and improvement of quality of life (QoL) is a critical objective in the therapy of patients with symptomatic advanced soft-tissue sarcoma (STS). The combination of trabectedin (T) and low-dose radiotherapy (RT) showed unprecedent activity in a previous study showing an overall response rate (ORR) of 60% according to RECIST by central radiological review. This study further explores this combination, with the aim of analyzing the QoL improvement in sarcoma patients.

Methods: Pattients with pretreated advanced/unresectable STS and symptomatic lesions were eligible to receive Trabectedin 1.5mg/m2 every 24h and RT 30Gy in 10 fractions (or 45 Gy in 25 fractions if the lesion was in limbs) on the symptomatic lesion/s, and then continue with Trabectedin up to progression or unacceptable toxicity. Primary end-point was ORR by RECIST on the irradiated lesion. Secondary endpoints included QoL assessments (Brief pain inventory (BPI), EORTC QLQ C30), progression-free survival (PFS) and overall survival (OS). With H0 (10%), H1 (35%), (α 0.05 and β error 0.1), 27 evaluable patients were necessary.

Results: From June/2021 to March/2023, 33 patients were included (18 Male/15 Female, median age 53 years (22-75), median previous lines 1 (0-4), ECOG 0/1 in 15/18 patients) and

26 patients were evaluable for response (efficacy population). Among the 26 evaluable patients, ORR in the irradiated lesions according to RECIST and local assessment was 42.3% (1 Complete Response, 10 Partial Responses, 13 Stable Disease, 2 Progressive Disease). With a median FU of 7 months (95% CI 1-13), 18 patients have progressed, with a median PFS of 5.2 months (95% CI 3.77-6.62) and 6m-PFS 44% (95% CI 23-64). During therapy, 17/33 (55%) of patients experienced a decrease of at least 2 points in the worst pain assessment of the BPI, with a median reduction of 2 points (range 0-8). 20/33 (64%) patients and 16/33 (52%) of patients reported an improvement of at least 1 point (on a scale 1-7) in their global health assessment and in their own QoL evaluation respectively. The main treatment-related G3-4 toxicities were neutropenia (18.2%), febrile neutropenia (9.1%), and ALT increased (9.1%). No toxic deaths have occurred.

Conclusion: Trabectedin plus palliative RT is a safe and active therapeutic regimen, able to induce clinical improvement in more than half of symptomatic sarcoma patients, and with a manageable toxicity profile.

(P 176) SAFETY, EFFICACY, AND PATIENT-REPORTED OUTCOMES WITH VIMSELTINIB IN PATIENTS WITH TENOSYNOVIAL GIANT CELL TUMOR WHO RECEIVED NO PRIOR ANTI–COLONY-STIMULATING FACTOR 1 THERAPY: ONGOING PHASE 2 UPDATE

Jean-Yves Blay, MD, PhD[1]; Piotr Rutkowski, MD, PhD[2]; Hans Gelderblom, MD, PhD[3]; Silvia Stacchiotti, MD[4]; Alejandro Falcon Gonzalez, MD[5]; Jayesh Desai, MBBS FRACP[6]; Axel Le Cesne, MD[7]; César Serrano, MD, PhD[8]; Virginia Ferraresi, MD[9]; Emanuela Palmerini, MD, PhD[10]; Breelyn A. Wilky, MD[11]; Andrew J. Wagner, MD, PhD[12]; Nicholas Bernthal, MD[13]; Michiel van de Sande, MD, PhD[3]; Supraja Narasimhan, MS, PhD[14]; Brooke S. Harrow, PhD[15]; Maitreyi G. Sharma, MD[14]; Rodrigo Ruiz-Soto, MD/MSc[14]; Matthew L. Sherman, MD[14]; William D. Tap, MD[16], (1) Centre Léon-Bérard, Lyon, Rhone-Alpes, France, (2) Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Mazowieckie, Poland, (3) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (4) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (5) Hospital Universitario Virgen del Rocío Sevilla and Instituto de BioMedicina de Sevilla, Seville, Andalucia, Spain, (6) Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia, (7) Gustave Roussy Institute, Villejuif, Ile-de-France, France, (8) Medical Oncology Department, Hospital Universitario Vall d'Hebron, Vall d'Hebron Institute of Oncology, Barcelona, Spain, Barcelona, Catalonia, Spain, (9) IRCCS Istituto Nazionale Tumori Regina Elena, Rome, Lazio, Italy, (10) IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (11) University of Colorado Cancer Center, Aurora, CO, (12) Dana-Farber Cancer Institute, Boston, MA, (13) University of California Los Angeles, Los Angeles, CA, (14) Deciphera Pharmaceuticals, LLC, Waltham, MA, (15) Deciphera Pharmaceuticals, LLC, WALTHAM, MA, (16) Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Tenosynovial giant cell tumor (TGCT) is a rare, locally aggressive neoplasm caused by upregulation of the colony-stimulating factor 1 (CSF1) gene, resulting in aberrant CSF1 expression and recruitment of CSF1 receptor (CSF1R)-dependent inflammatory macrophages. Vimseltinib is an investigational, oral switch-control tyrosine kinase inhibitor specifically designed to selectively and potently inhibit CSF1R. Here, we report updated safety, efficacy, and patient-reported outcome (PRO) data from Cohort A of the phase 2 part (expansion) of an

ongoing phase 1/2 study (NCT03069469) for patients (pts) with TGCT treated with vimseltinib who did not receive prior specific anti-CSF1/CSF1R agents.

Methods: Pts with TGCT not amenable to surgery and no prior anti-CSF1/CSF1R therapy (previous treatment with imatinib or nilotinib was allowed) were enrolled and treated with vimseltinib 30 mg twice weekly (recommended phase 2 dose). The primary objectives for Cohort A were to evaluate safety, tolerability, and antitumor activity (by independent radiological review [IRR] using Response Evaluation Criteria in Solid Tumors version 1.1 [RECIST v1.1]). PROs (secondary objectives) were also evaluated and reported for the 25-week timepoint. Pain was assessed by 2 items from the brief pain inventory (BPI; worst pain and average pain [baseline: average from a 2-week period during screening; week 25: average from weeks 2 and 3 of month 6]) and compared with tumor responses at 25 weeks (by IRR using RECIST v1.1).

Results: As of Jan 27, 2023, 46 pts with TGCT with no prior anti-CSF1/CSF1R therapy were enrolled (enrollment complete; Table). Median age was 44 years (range, 21–71), and the most common tumor location was the knee (57%; 26/46). The safety profile remained consistent with prior reports. Most nonlaboratory treatment-emergent adverse events (TEAEs) were grade 1/2; grade 3/4 TEAEs (≥5% of pts) were elevated creatine phosphokinase and hypertension. There were no treatment-related serious adverse events and no evidence of cholestatic hepatotoxicity. TEAEs led to dose reductions and interruptions in 50% (23/46) and 65% (30/46) of pts, respectively. The median treatment duration was 15.9 months (range, 0.2–24.9; mean, 13.4 months); 52% (24/46) of pts were still on treatment at the time of this analysis. Reasons for treatment discontinuation included adverse events (n=3), physician decision (n=6), and withdrawal by pt (n=13). No pts progressed as assessed by IRR using RECIST v1.1 (median follow-up from first dose, 16.7 months). The objective response rate (ORR) was 60% (27/45; Table 1). The majority of responses (67%; 18/27) were achieved within 6 months after treatment initiation, with a median time to first response of 3.7 months (range, 1.8–22.2). At week 25, 48% (22/46) and 52% (24/46) of pts demonstrated a ≥30% reduction in BPI worst pain and average pain, respectively. Most pts who had a tumor response at week 25 also experienced ≥30% reductions in BPI worst pain and average pain (among responders with BPI data available at week 25; Table 2). Pts with stable disease (SD) at week 25 also experienced ≥30% reductions in BPI worst pain and average pain.

Conclusion: Longer follow-up demonstrated that vimseltinib continued to be well tolerated with a manageable safety profile in pts with TGCT not amenable to surgery with no prior anti-CSF1/CSF1R therapy. Antitumor activity continued to improve with a promising overall ORR. At week 25, the majority of responders experienced ≥30% reductions in worst and average pain. Pain reductions were also observed in pts with SD, suggesting that vimseltinib provides pain relief with or without an objective tumor response. Taken together, these data demonstrate that vimseltinib can provide antitumor activity and pain relief in pts with TGCT.

Table 1. Response as assessed by IRR using RECIST v1.1 in patients with TGCT receiving vimseltinib

	Patients with no prior anti-CSF1/CSF1R therapy
Safety population, n	46
Efficacy-evaluable population, n	45
Best overall response, n (%)[a]	
Objective response rate	27 (60)
Complete response	0
Partial response	27 (60)
Stable disease	18 (40)
Duration of response, median[b] (min, max), months	NR (0.03+, 20.3+)

+ indicates patients are ongoing on study.
[a]Best overall response by IRR using RECIST v1.1; includes all available follow-up.
[b]Based on Kaplan-Meier estimate. Duration of response is defined as time from first imaging result showing response to progressive disease.
CSF1, colony-stimulating factor 1; CSF1R, CSF1 receptor; IRR, independent radiological review; max, maximum; min, minimum; NR, not reached; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; TGCT, tenosynovial giant cell tumor.

Table 2. BPI worst pain and average pain correlated with tumor response at week 25 in patients with TGCT receiving vimseltinib

≥30% pain reduction at week 25, n (%)	Tumor response at week 25[a]	
	Partial response n = 14	Stable disease n = 22
Worst pain	10 (71)	12 (55)
Average pain	12 (86)	12 (55)

Includes patients with both BPI and tumor response data available at week 25 (n = 36); percentages represent proportion of patients with partial response or stable disease with ≥30% pain reduction.
[a]By IRR using RECIST v1.1.
BPI, Brief Pain Inventory; IRR, independent radiological review; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; TGCT, tenosynovial giant cell tumor.

(P 177) ANGIOSARCOMA: AN INCREASING PROBLEM IN THE UNITED STATES

Michael J. Wagner, MD[1]; Vinod Ravi, MD, MS, MBA[2]; Stephanie K. Schaub, MD[3]; Edward Kim, MD[3]; Jeremy Sharib, MD[3]; Harveshp Mogal, MD[3]; Min Park, M.D.[4]; Daniela Duarte-Bateman, MD[5]; Anthony Tufaro, M.D.[5]; Elizabeth T. T. Loggers, MD, PhD[6]; Lee D. Cranmer,

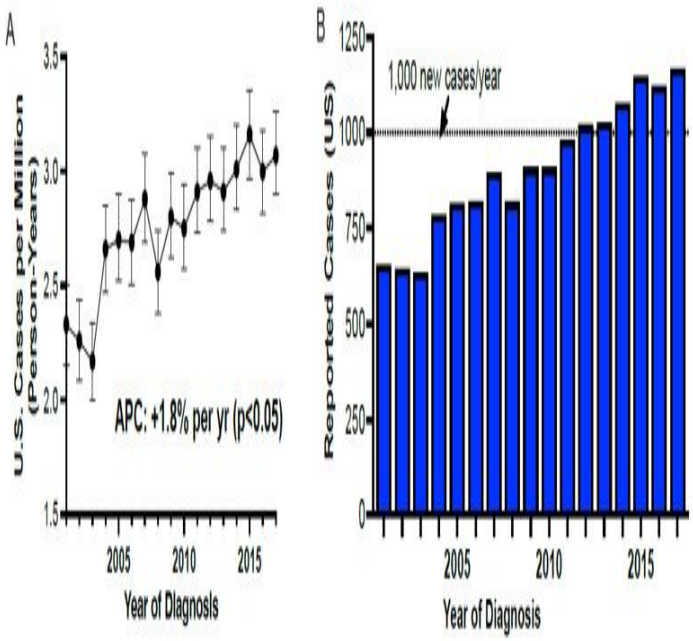
MD, PhD, F.A.C.P.[7]; Bonny Chau, MPH[3]; Michael Hassett, M.D., MPH[8]; Juneko Grilley-Olson, MD[9]; Kelly G. Paulson, M.D.[4], (1) Dana Farber Cancer Institute , Boston, MA, (2) The University of Texas MD Anderson Cancer Center, Houston, TX, (3) University of Washington, Seattle, WA, (4) Swedish Cancer Institute, Seattle, WA, (5) Cleveland Clinic, Cleveland, OH, (6) Fred Hutch Cancer Center, Seattle, WA, (7) University of Washington/Fred Hutchinson Cancer Center, Seattle, WA, (8) Dana Farber Cancer Institute, Boston, MA, (9) Duke University, Durham, NC

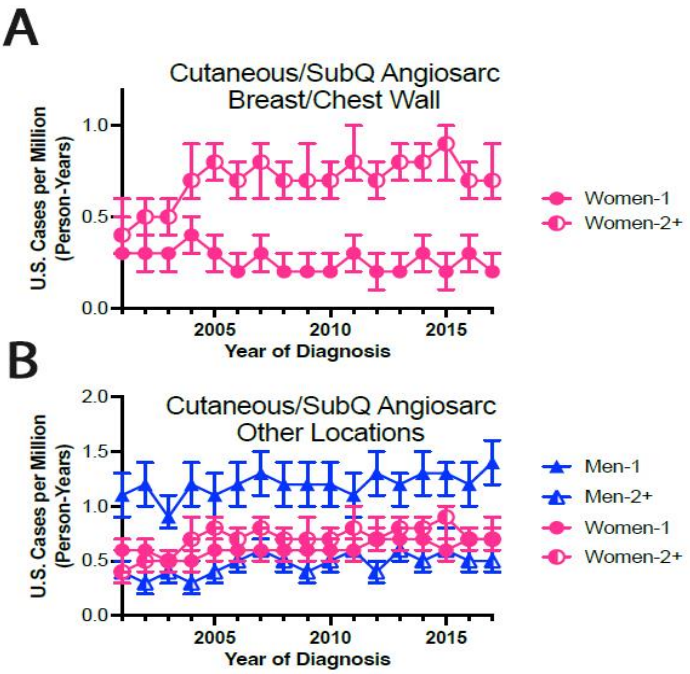
Objective: Angiosarcoma is an aggressive cancer of endothelial cells that can arise in any part of the body, but most commonly occurs in the skin. Angiosarcoma can form without a clear inciting factor or arise as a secondary malignancy after radiation or from chronic lymphedema. Although rare, our prior work suggested that the incidence of angiosarcoma may be rising in the United States (US). We sought to describe the incidence, presenting characteristics, and change over time of angiosarcoma in the US.

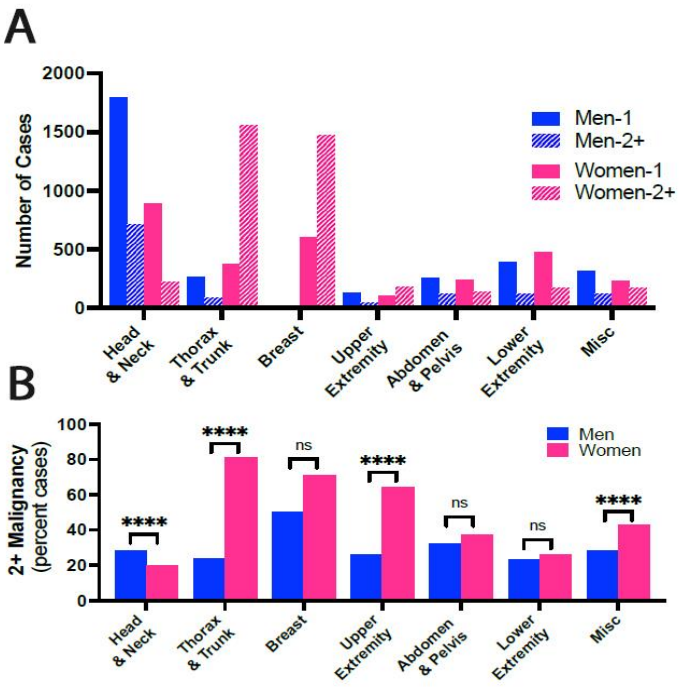
Methods: A retrospective cross-sectional database analysis was conducted utilizing the US Cancer Statistics NPCR-SEER Combined Database (USCS), which captures >99% of newly diagnosed US cancers. All US, newly diagnosed angiosarcoma patients between the years of 2001-2017 (n=15,410) were included. Main outcomes were incidence, patient demographics including history of prior cancer before angiosarcoma diagnosis, and extent of disease at presentation. Changes in rates were determined with age-adjustment to the 2000 US Standard Population; percent changes were calculated using the weighted least-squares method.

Results: The US incidence of angiosarcoma nearly doubled between 2001 (653 cases) and 2017 (1166 cases). This corresponded to a rise in population adjusted incidence rate of 1.8% per year ($p< 0.01$), to a current rate of 3.1 cases per million person-years (95% CI 2.9-3.3) and rise in population at risk (Figure 1). Secondary breast and chest wall angiosarcomas in women represented the largest contribution to increasing incidence (Figure 2). Women were more likely to have secondary cutaneous angiosarcoma of the thorax and upper extremity (80.5% of chest wall/thorax cases in women were secondary vs 24.2% in men and 63.9% of upper extremity cases in women were secondary vs 25.7% in men, $p < 0.05$), compared to men who were more likely to have secondary angiosarcoma of the head or neck (28.3% vs 19.6%, $p< 0.05$, Figure 3). Overall, 72.6% were cutaneous/subq/breast (n=11,187), 22.9% were visceral (n=3,523), and 4.5% were unknown or rare primary sites (n=700). Median age at diagnosis was ~65 years for primary cutaneous angiosarcoma and visceral angiosarcomas, and ~75 years for secondary cutaneous angiosarcoma. Almost all cases in the breast were in women (99.2%), 70.4% of breast angiosarcomas were secondary. At presentation, 60% of primary angiosarcomas and 73% of secondary angiosarcomas presented with local or regional disease. The incidence rate of visceral angiosarcoma is also increasing (1.8% per year; $p < 0.01$). The most common sites of visceral primary disease were liver (27.5%), heart (17.3%) and bone (12.5%).

Conclusion: This study describes angiosarcoma presentation patterns and incidence in the United States over a 17-year period and shows that the number of cases is increasing, with the greatest rise in women with secondary angiosarcoma of the chest/breast/upper extremity. These data increase awareness of a rare but highly morbid disease and highlight the need for monitoring and increased suspicion of angiosarcoma in patients at higher risk, such as women who have a prior history of breast cancer.


A
U.S. Cases per Million (Person-Years)
3.5
3.0
2.5
2.0
1.5
APC: +1.8% per yr (p<0.05)
2005
2010
2015
Year of Diagnosis
B
Reported Cases (US)
1250
1000
750
500
250
0
1,000 new cases/year
2005
2010
2015
Year of Diagnosis


A
Cutaneous/SubQ Angiosarc
Breast/Chest Wall
U.S. Cases per Million (Person-Years)
1.0
0.5
0.0
2005
2010
2015
Year of Diagnosis
Women-1
Women-2+
B
Cutaneous/SubQ Angiosarc
Other Locations
U.S. Cases per Million (Person-Years)
2.0
1.5
1.0
0.5
0.0
2005
2010
2015
Year of Diagnosis
Men-1
Men-2+
Women-1
Women-2+



(P 178) INITIAL RESULTS FROM A PHASE 1 STUDY OF CFT8634, A NOVEL BIFUCTIONAL DEGRADATION ACTIVATING COMPOUND (BIDAC) DEGRADER OF BRD9, IN SYNOVIAL SARCOMA AND SMARCB1-NULL TUMORS

Mark Agulnik, MD[1]; William D. Tap, MD[2]; Gregory M. Cote, MD, PhD[3]; Ravin Ratan, MD[4]; Shaheer Khan, D.O[5]; Sant P. Chawla, MD, FRACP[6]; Steven Attia, D.O.[7]; Anthony D. Elias, MD[8]; Mohammed Milhem, MBBS[9]; Joseph Pressey, MD[10]; Ingrid Mintautas, B.A[11]; Riadh Lobbardi, PhD[12]; Amro Ali, PharmD[13]; Huan Liu, M.A[12]; Eunju Hurh, PhD[12]; Kathleen Neville, MD[12]; Brian Andrew Van Tine, MD, PhD[14], (1) City of Hope, Department of Medical Oncology & Therapeutics Research, Duarte, CA, (2) Memorial Sloan Kettering Cancer Center, New York, NY, (3) Mass General Cancer Center, Boston, MA, (4) The University of Texas MD Anderson Cancer Center, Houston, TX, (5) 5Columbia University Herbert Irving Comprehensive Cancer Center, New Yor, NY, (6) Sarcoma Oncology Center, Santa Monica, CA, (7) Mayo Clinic, Jacksonville, FL, (8) University of Colorado Anschutz Medical Campus, Aurora, CO, (9) University of Iowa, Holden Comprehensive Cancer Center, Iowa City, IA, (10) Cincinnati Children's Hospital, Cincinnati, OH, (11) C4 Therapeutics, Chicago, IL, (12) C4 Therapeutics, Watertown, MA, (13) C4 Therapeutics, Stoneham, MA, (14) Washington University School of Medicine, St. Louis, MO

Objective: CFT8634 is an orally bioavailable, selective, bifunctional degradation activating compound, or BiDAC™ degrader, of the Bromodomain-containing protein 9 (BRD9). BRD9 belongs to the non-canonical SWI/SNF complex and is essential to the proliferation of SMARCB1-perturbed cancers. These include synovial sarcoma, defined by the SS18-SSX fusion, and SMARCB1-null tumors such as epithelioid sarcoma. Synovial sarcoma is a rare soft tissue malignancy comprising ~10% of all soft tissue sarcomas. In the metastatic setting,

therapeutic options are limited and outcomes are poor with a median overall survival of 17.0 months and 1-year survival rate of 59.7%. CFT8634 showed a robust and dose-dependent BRD9 degradation in preclinical tumor models of synovial sarcoma and SMARCB1-null tumor models and anti-tumor activity in patient-derived xenograft models of synovial sarcoma. Here we report pharmacokinetics (PK), pharmacodynamics (PD), and safety data from the first four cohorts of the CFT8634-1101 phase 1 trial where CFT8634 is being evaluated in patients with synovial sarcoma and SMARCB1-null tumors.

Methods: CFT8634-1101 (NCT05355753) is an ongoing first-in-human, open-label, multicenter, phase 1 dose-escalation and cohort-expansion (phase 2) clinical trial. Phase 1 primary objectives include safety, tolerability and RP2D/MTD of CFT8634. Secondary objectives include assessment of PK, PD , and preliminary efficacy endpoints of CFT8634. Key inclusion criteria are histologically or cytologically confirmed synovial sarcoma or SMARCB1-null tumors (SMARCB1 status ascertained by immunohistochemistry (IHC), fluorescent in situ hybridization, mutation analysis or other diagnostic tests), with unresectable or metastatic disease, following at least 1 prior line of standard-of-care systemic therapy.

Pharmacokinetic parameters on Cycle 1 Day 1 and Cycle 1 Day 15 were calculated using non-compartmental methods. Level of BRD9 degradation upon CFT8634 dosing were assessed by BRD9 IHC in tumor tissue at baseline and Cycle 1 Day 15 (on treatment biopsy) and by mass spectrometry in peripheral blood mononuclear cells at various timepoints.

Results: As of 17 May 2023, a total of 26 patients have been treated with CFT8634 in the 2 mg (n=2), 4 mg (n=5), 8 mg (n=6), 15 mg (n=7), and 30 mg (n=6) cohorts. Patients enrolled in the 30 mg cohort are under evaluation at the time of the data cutoff and are excluded from this analysis. From the first four dose cohorts, the median age was 37 (19-62) years, and most patients (85%) received at least 3 prior lines of therapy, ranging from one prior line to >4 prior lines of therapy. No dose limiting toxicities (DLTs) have been observed at any of the dose levels. The most common and predominately low-grade TEAEs (≥20%) include dry mouth (40%), anemia (30%), dysgeusia (30%), fatigue (30%), and neutropenia (25%). The most common ≥G3 TEAE (≥10%) observed was hyperbilirubinemia (10%). At the time of the data cutoff, there were two subjects who had dose escalated from 8 mg to 15 mg.

Plasma exposure of CFT8634 increased proportionally with dose after single- and repeat-dose administration and a half-life of 10-14 hours supports once-daily dosing. Pharmacodynamic data show a rapid (as early as 8 hours post first dose), robust and sustained BRD9 degradation, assessed in blood and tumor tissue, in most patients at different dose levels of CFT8634. These data confirmed CFT8634 is a potent BRD9 degrader as shown in the preclinical observations and analyses using synovial sarcoma and SMARCB1-loss in in vitro and in vivo models.

Conclusion: Preliminary findings from CFT8634-1101 indicate that CFT8634 demonstrated favorable tolerability and safety at early dose levels. Updated data to be presented at the conference.

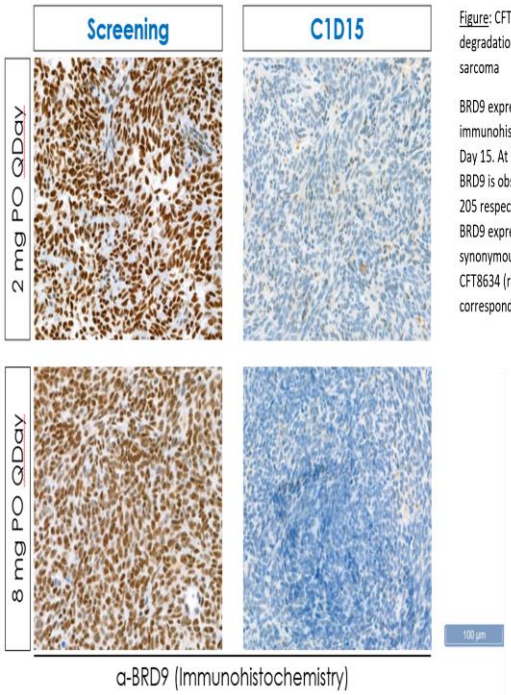


Figure: CFT8634 shows high level of BRD9 degradation at Cycle 1 Day 15 in synovial sarcoma

BRD9 expression is assessed by immunohistochemistry at Screening and Cycle 1 Day 15. At 2mg and 8mg, strong expression of BRD9 is observed (left panels; H-score: 240 and 205 respectively). At Cycle 1 Day 15, a lack of BRD9 expression is observed for both doses, synonymous of potent degradation activity by CFT8634 (right panels; H-score: 40 and 35 corresponding to 83.3% and 82.9% respectively)

(P 179) PILOT STUDY OF A REMOTELY DELIVERED RESISTANCE EXERCISE TRAINING PROGRAM IN SARCOMA SURVIVORS

Lara Davis, MD[1]; Shannon Austin, MS[2]; James Hayden, MD, PhD[3]; Yee-Cheen Doung, MD[3]; Kenneth R. Gundle, MD[4]; Christopher W. Ryan, MD[5]; Sydnee A. Stoyles, MBST[3]; Kerri Winters-Stone, PhD[3], (1) OHSU Knight Cancer Institute, Portland, OR, (2) Oregon State University, Tualatin, OR, (3) Oregon Health & Science University, Portland, OR, (4) Portland VA Medical Center, Portland, OR, (5) OHSU Knight Cancer Institute, South Waterfront, Portland, OR

Objective: Treatment of sarcoma may lead to serious long-term health consequences, including decreased physical functioning. Supervised resistance training may improve physical functioning in sarcoma survivors as long as it is accessible and adequately tailored to each patient's abilities. We conducted a single-arm, pre-post study to assess the feasibility of remote delivery (through videoconferencing technology) of a supervised, individualized resistance training program in post-treatment sarcoma survivors.

Methods: Eligible patients were at least 15 years of age and at least 2 years from end of treatment for a sarcoma of the extremity, axial skeleton, or body wall (intra-thoracic, intra-abdominal, retroperitoneal and cranial not eligible). Participants followed a progressive resistance training (PRT) program of prescribed exercises that could be tailored (e.g., alternate exercises and/or adjusted volume) based on the patient's initial exercise capacity. Participants engaged in one supervised session delivered remotely by video conferencing and one unsupervised session per week for 12 weeks. Feasibility was measured by 12-week retention rate, session completion rate, and safety. Changes in self-reported physical function, health-related quality of life, and fatigue were assessed with PROMIS, SF-36 and FACIT-F

questionnaires. Objective physical function was to be assessed by the short physical performance battery (SPPB), but the COVID pandemic required protocol modification to objective assessments.

Results: Ten participants enrolled in the study and 9 completed end-of-intervention assessments (90% retention). Median age 47 (range 27-69), and subjects were a median of 4 years (range 2-16) from end of sarcoma treatment. Half (5) had a history of bone sarcoma. All participants had undergone surgery and chemotherapy for treatment, and 50% had also received radiation. Adherence to twice-weekly exercise sessions was 78% and no related serious adverse events occurred. Patients who completed the intervention reported improvements in physical functioning, health-related quality of life and fatigue. Significant improvements in SF-36 physical role and bodily pain subscales and physical component summary scores all exceeded minimal clinically important differences in pre-post scores, with moderate-to-large effect size (d>0.50). Self-reported fatigue also significantly improved (FACIT-Fatigue d=0.61, p=0.06). SPPB data was incomplete due to COVID-related study disruption of in-person testing.

Conclusion: Remote delivery of PRT was feasible and safe in sarcoma survivors and improved several domains of health-related quality of life. These data suggest that remote exercise training could be adequately scaled and delivered in a larger, definitive exercise trial. Such a trial could inform specific exercise prescription(s) for sarcoma patients, who currently have little evidence-based exercise guidance. (Supported by P30CA069533. ClinicalTrials.gov NCT04247425)

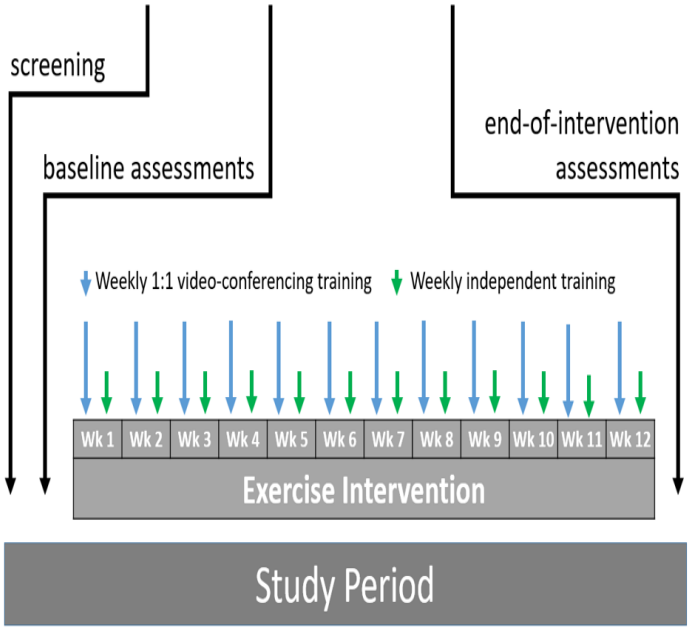


Figure 1. Schematic of study design.

Table 1. Initial PRT prescription. This initial prescription is modified by EP to account for individual physical limitations, to provide a tailored PRT prescription.

Activity Type	Prescription				Schedule
	Sets	*Reps*	*Time*	*Resistance*	
Warm-up			10min		2 times per week (once with EP and once independently)
Squat	2-3	10-15		Body weight	
Chest Press	2-3	10-15		Band	
Bent-Knee Deadlift	2-3	10-15		Band	
Side Step	2-3	10-15		Band	
Lunge w/ One-Arm Row	2-3	10-15		Body weight + band	
Reverse Lunge with Kick	2-3	10-15		Body weight + band	
Push Up (modified)	2-3	10-15		Body weight	
Plank	2-3	10-15	20s-1min	Body weight	
Glute Bridge	2-3	10-15		Body weight	
Cool down			8-10min		

(P 180) HEALTH RELATED QUALITY OF LIFE OF PATIENTS WITH ADVANCED LEIOMYOSARCOMA AND OTHER SOFT TISSUE SARCOMAS TREATED WITH CABOZANTINIB AND TEMOZOLOMIDE

Varun Monga, MBBS[1]; Mohammed Milhem, MBBS[2]; John Charlson, MD[3]; Oluwatimilehin Okunowo, MPH[4]; Paul Frankel, MD[4]; Brian Schulte, MD[1]; Brian Van Tine, MD[5]; Angela Hirbe, MD,PHD[5]; Mia C. Weiss, MD[6]; Scott Okuno, M.D.[7]; Brittany Siontis, MD[7]; Steven Attia, D.O.[8]; Steven Robinson, MBBS[7]; Seth Pollack, MD[9]; Pedro Viveiros, MD[10]; Janet Yoon, MD[4]; Mark Agulnik, MD[11], (1) UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, (2) University of Iowa, Holden Comprehensive Cancer Center, Iowa City, IA, (3) Medical College of Wisconsin, Wauwatosa, WI, (4) City of Hope Comprehensive Cancer Center, Duarte, CA, (5) Washington University in St. Louis, St. Louis, MO, (6) Washington University School of Medicine, St. Louis, MO, (7) Mayo Clinic, Rochester, MN, (8) Mayo Clinic, Jacksonville, FL, (9) Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL, (10) Northwestern University, Chicago, IL, (11) City of Hope, Department of Medical Oncology & Therapeutics Research, Duarte, CA

Objective: Oral combination strategies are increasingly being studied in management of advanced soft tissue sarcomas but may be associated with increased toxicities. Patient reported outcomes (PROs) provide necessary information on toxicities of a given regimen from a patient perspective and help guide management. Cabozantinib (CAB), an oral tyrosine kinase inhibitor targeting dual VEGF and c-MET pathways was combined with temozolomide (TMZ), an alkylating agent in a Phase II trial in patients with advanced leiomyosarcoma (cohort 1) and other soft tissue sarcomas (cohort 2) which showed improvement in PFR at 12 weeks > 39%. Pooled PRO data across cohorts was collected at scheduled time points during the study and

are reported.

Methods: Across 5 sites included in the Midwest Sarcoma Trials Partnership a total of 72 patients were enrolled (42 in cohort 1 and 30 in cohort 2). Inclusion criteria included age ≥ 18, adequate performance status (ECOG 0-1), organ function, measurable disease (RECIST 1.1), and 0-5 prior chemotherapy regimens. CAB 40 mg PO daily plus TMZ 150-200 mg/m2 PO 1-5 days were given in 28-day cycles until disease progression or unacceptable toxicity. Participants completed the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30, which is scored 0-100) at baseline during screening and then on Day 1 of every cycle prior to the study treatment administration, and at the end of treatment. A linear mixed effects model was used to assess EORTC QLQ-C30 trends over time. Trends were assessed from baseline to Cycle 3 (~ 12 weeks). The analyses were performed for the following covariates: response group (responders and non-responders), age group (< 40, 40-60, and >60 years), and gender (female and male). An interaction term of the covariates with time was included in the model to explore if the longitudinal trajectories differed by levels of the covariates. P-values < .05 were considered statistically significant and all tests were 2-sided. Data management and statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 4.3.0 (R Foundation for Statistical Computing).

Results: 63/72 (88%) patients completed a baseline QOL survey and 43 (60%) patients completed the QOL survey at all predefined points up to cycle 3 in the study. ECOG performance score of 0 was present in 37/63 (59%) and 26/43 (60%), respectively. 6/63 patients had a partial response as per RECIST 1.1 criteria and all 6 responders were included in the 43 patient subset as well. While baseline ECOG 0 percentage was slightly higher in responders (67% vs 58%, p=NS), at baseline, responders had higher mean QLQ-C30 scores of physical functioning (92.8 vs 79.6, p =0.003), role functioning (94.4 vs 76.0 p=0.020) and global health status score (80.6 vs 69.0, p=0.001). Appetite loss (5.6 vs 22.2, p=0.033) and financial problems (5.6 vs 22.8, p=0.029) were reduced in responders when compared to non-responders. When restricted to the 43 patients with 3-cycles of QOL data, Overall the estimated EORTC QLQ-C30 symptom scores for diarrhea, nausea/vomiting, fatigue and appetite loss were reported to increase over time but there was no significant interaction effect based on clinical response, age groups or gender. Constipation symptoms did not statistically change over time.

Conclusion: Patients with advanced leiomyosarcoma and soft tissue sarcoma with higher baseline QLQ-C30 scores of physical functioning, role functioning, global health status scores and less appetite loss or financial problems during treatment with CAB + TMZ had better clinical responses. Diarrhea and nausea/vomiting worsened over the first 3 cycles, suggesting management during the course of treatment may need to intensify. Fatigue and appetite loss also worsened. The reported symptoms are commonly seen with use of either drug hence may need careful monitoring and management of toxicities to best treat patients with this regimen.

(P 181) REAL-WORLD EXPERIENCE WITH LIPOSOMAL DOXORUBICIN IN METASTATIC AND LOCALLY ADVANCED SARCOMA

Allen Hodge, MD[1]; Meena Bedi, MD, MS[2]; Alexandra C. Istl, MD, MPH[2]; Callisia Clarke, MD[3]; David King, MD[2]; John C. Neilson, MD[2]; Adam Wooldridge, MD[2]; John Charlson, MD[4], (1) Massachusetts General, Boston, MA, (2) Medical College of Wisconsin, Milwaukee, WI, (3)

Medical College Of Wisconsin - Surgical Oncology, Milwaukee, WI, (4) Medical College of Wisconsin, Wauwatosa, WI

Objective: Doxorubicin is commonly used as first-line chemotherapy in metastatic sarcoma; pegylated liposomal doxorubicin (PLD) may be considered as a potentially better tolerated alternative to free doxorubicin, with less myelosuppression, alopecia, and cardiotoxicity. In this project, we examined the efficacy and toxicity associated with using PLD as treatment for metastatic and locally advanced soft tissue and bone sarcoma.

Methods: Medical records of metastatic and locally advanced sarcoma patients treated with single agent PLD were reviewed at a single institution. Retrospective analysis was conducted to measure overall survival, progression free survival, dosage, and complications. RECIST criteria were applied retrospectively to routine imaging studies to estimate response and disease progression endpoints.

Results: 58 subjects who completed the planned treatment and had imaging appropriate for response evaluation were included in the analysis. Mean age at time of initiating therapy was 64 years (range 33-84 years). Sarcoma subtypes included liposarcoma (28%), leiomyosarcoma (21%), undifferentiated sarcoma (16%), synovial sarcoma (5%), and other subtypes (30%). 22% of patients had previously been treated with doxorubicin, in the neo-adjuvant or adjuvant setting. Patients received an average of 5 cycles of PLD. Starting dosage was 50 mg/m² every 28 days, however, reductions in the starting dose were made in 38% of cases due to anticipated tolerance, and in 21% subsequently due to side effects such as cutaneous toxicity and fatigue. Of 110 sarcoma patients initially identified as been treated with PLD, 8 were excluded from analysis because treatment was discontinued early due to infusion reaction. There were no complete responses, 12% partial response, 31% stable disease, and 57% progressive disease. Six of 18 patients with SD had PFS greater than 180 days. Mean overall survival 452 days. Mean and median PFS was 180 days and 89 days, respectively. Sixty-seven percent of participants had at least one follow-up echocardiogram, and 15% of those had some evidence of cardiotoxicity, with an ejection fraction of < 50% or 10% decrease from baseline. Of the participants with changes in ejection fraction, 67% had received prior anthracycline therapy and 33% had received a total lifetime dose of doxorubicin exceeding the recommended 500 mg/m² limit.

Conclusion: PLD is an option for treatment of advanced sarcoma, with a 12% response rate and mean progression free survival of 180 days. PLD is generally well-tolerated in an older population, but there are potential toxicities that require attention, including cutaneous toxicity and infusion reactions. A notable portion of our sample had echocardiographic changes, with decreased ejection fraction, highlighting the fact that, while PLD may be less cardiotoxic than free doxorubicin, there is potential cardiac risk.

(P 182) UPDATED RESULTS FROM AN ONGOING PHASE 1B STUDY OF UNESBULIN (PTC596) PLUS DACARBAZINE FOR THE TREATMENT OF PATIENTS WITH ADVANCED LEIOMYOSARCOMA

Brian Andrew Van Tine, MD, PhD[1]; Matthew A. Ingham, MD[2]; Steven Attia, D.O.[3]; Christian F. Meyer, MD PhD[4]; John D. Baird, PhD[5]; Dhiren D'Silva, MBA[5]; Kylie O'Keefe[5]; Mark Rance, MBBS, MRCS[5]; Marla Weetall, PhD[5]; Gary K. Schwartz, MD[6], (1) Washington University School of Medicine, St. Louis, MO, (2) Columbia University Medical Center, New York, NY, (3) Mayo Clinic, Jacksonville, FL, (4) Johns Hopkins Sidney Kimmel Comprehensive Cancer

Center, Baltimore, MD, (5) PTC Therapeutics, Inc., South Plainfield, NJ, (6) Herbert Irving Comprehensive Cancer Center at Columbia University School of Medicine, New York, NY

Objective: Leiomyosarcoma (LMS) is one of the most common subtypes of soft tissue sarcoma and is associated with a high risk of relapse and a poor prognosis for advanced disease. In preclinical LMS models, unesbulin, a microtubule polymerization inhibitor, potentiated the activity of dacarbazine (DTIC) (Jernigan F, et al. Mol Cancer Ther. 2021;20:1846–1857). Here, we report updated safety and efficacy results from a Phase 1b dose escalation study evaluating the combination of unesbulin with DTIC in patients with advanced LMS (NCT03761095).

Methods: In this single-arm, open-label, Phase 1b clinical trial, patients with advanced LMS received unesbulin orally at 200, 300, or 400 mg twice weekly (BIW) in combination with intravenous DTIC at 1,000 mg/m2 once every 21 days. The primary objectives were to determine the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) of unesbulin in combination with DTIC and to characterize the safety profile of the combination. The secondary objectives include the evaluation of antitumor activity of unesbulin in combination with DTIC. The time-to-event continual reassessment model was used for dose finding. A total of 41 patients were enrolled, which included 12 patients in a food-effect expansion cohort.

Results: As of the data cutoff on May 8th, 2023, 41 patients with LMS have been treated. Median prior lines of therapy were 3 with 19 patients (46.3%) receiving 4 or more prior lines of therapy. Twenty patients had non-uterine and 21 patients had uterine LMS. Thirty-three patients received therapy at the RP2D, that was previously determined as 300 mg BIW with DTIC 1,000 mg/m2 every 21 days. The most common treatment-related adverse events included fatigue, diarrhea, anemia, decrease in appetite, nausea, vomiting, and decrease in platelet, neutrophil, white blood cell, and lymphocyte counts. In the patients evaluable for efficacy, the objective response rate (ORR) was 21.6% (8/37) and the disease control rate (DCR) (DCR = complete response + partial response + stable disease at 12 weeks) was 54.1%. At the 300 mg dose level, the ORR was 24.1% (7/29) and the DCR was 55.2%. Patients received a median of five cycles (range 1–28). The study is ongoing, with patients continuing to receive treatment. Updated clinical results will be presented at the 2023 Connective Tissue Oncology Society Congress in Dublin, Ireland.

Conclusion: Unesbulin 300 mg BIW in combination with DTIC 1,000 mg/m2 every 21 days was well tolerated and demonstrated promising efficacy in a heavily pre-treated patient population with advanced LMS; these results support further investigation. A randomized, placebo-controlled, Phase 2/3 trial is ongoing (NCT05269355).

(P 183) A PHASE 1B/2 STUDY OF LIPOSOMAL ANNAMYCIN (ANN) IN SUBJECTS WITH PREVIOUSLY TREATED SOFT-TISSUE SARCOMAS (STS) WITH PULMONARY METASTASES

Brian Andrew Van Tine, MD, PhD[1]; Maria A. Zarzour, MD[2]; Sant P. Chawla, MD, FRACP[3]; David Liebner, MD[4]; Sarah Weiss, MD[5]; Cynthia Abbate, BS[6]; Robert Shepard, MD[7]; Erikson Wasyl, MS[8]; J Paul Waymack, MD[9]; Waldemar Priebe, PhD[10], (1) Washington University School of Medicine, St. Louis, MO, (2) MD Anderson Cancer Center, Houston, TX, (3) Sarcoma Oncology Center, Santa Monica, CA, (4) Ohio State University, James Cancer Hospital and Solove Research Center, Columbus, OH, (5) Rutgers Cancer Institute of New Jersey, New

Brunswick, NJ, (6) Moleculin Biotech, Inc, Stockton, NJ, (7) UNC, Houston, TX, (8) Moleculin Biotech, Inc., Durham, CT, (9) Moleculin Biotech, Inc, Houston, TX, (10) Experimental Therapeutics, MD Anderson Cancer Center/ Moleculin Biotech, Inc, Houston, TX

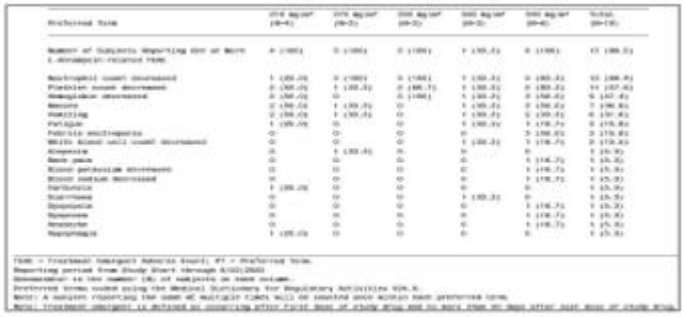
Objective: The most common site of STS metastases is the lungs. Doxorubicin (DOX) remains a mainstay for standard treatment, but clinical benefit is not prolonged and toxicities, especially cardiotoxicity, limit its use. L-Annamycin ("ANN" or "Annamycin")" is a multilamellar liposomal formulation active against multidrug-resistant tumors and does not elicit cardiotoxicity. As preclinical studies documented ANN localizes in pulmonary tissues at higher concentrations than DOX, it is an ideal candidate to explore in subjects with lung metastases. The primary objective of the Phase 1b portion of the study was to evaluate safety and to identify the maximum tolerated dose (MTD) and/or recommended Phase 2 dose (RP2D). The primary objective of Phase 2 was to assess efficacy, including, but not limited to, overall response rate (ORR), ORR targeted to pulmonary metastases, disease control rate (DCR), and progression-free and overall survival (PFS and OS, respectively).

Methods: ANN was administered on Day 1, followed by 20 days off. Safety monitoring included ECHOs, ECGs, cardiac biomarkers, and laboratory evaluations. Treatment continued every 21 days until disease progression, clinical deterioration, or unacceptable toxicity. Efficacy was monitored radiographically every 6 weeks while on therapy, then every 3 months. In Phase 1b, dose escalation proceeded according to a 3 + 3 design. During Phase 2, up to 25 subjects were to be enrolled at the RP2D. Eligible subjects were ≥18 years of age with a confirmed diagnosis of STS, unresectable, measurable disease in the lung, progression on prior therapy, and no significant comorbidities. Subjects evaluable for efficacy were assessed for response to treatment by radiologic imaging.

Results: 19 subjects were enrolled in the Phase 1b portion of the study. The median number of cycles administered was 2. There were no significant safety concerns or unexpected SAEs up to the 390 mg/m2 dose. The RP2D was defined as 330 mg/m2. The most frequently reported AEs related to ANN were in the system organ class of Investigations (Table 1). There was no evidence of cardiotoxicity as measured by ejection fraction, strain analyses, ECGs, and cardiac biomarkers including Troponin-I and T. To date in Phase 2, 11 subjects (median # of prior therapies = 3) have received at least 2 cycles of 330 mg/m2. Of these 11 subjects, 7 (64%) showed stable disease (SD) through 2 cycles (5 of whom continued to show SD through 4 cycles), and 2 (18%) showed SD through 5 cycles. Of these 2, one subject has maintained SD through the end of 6 cycles with the next imaging assessment expected at the end of cycle 8 (5.5 months). The other subject has had SD through 5 cycles with the next imaging assessment expected at the end of cycle 6 (4.1 months). 5 subjects continue to be followed for PFS, and all 11 subjects in the efficacy population are being followed for OS.

Conclusion: This trial has established the RP2D dosing regimen of ANN as 330 mg/m2. Preliminary efficacy findings at the RP2D are encouraging with a DCR of 64% at the end of cycle 2 in this heavily pretreated population. To date, there has been no evidence of cardiotoxicity. This further supports that ANN may have significant advantages over currently approved anthracyclines for which effectiveness and duration of treatment are hampered due to cardiotoxic properties. This Phase 2 trial will continue to enroll and follow patients to better quantify efficacy.

Table 1: L-Asparaginase-Related Adverse Events by Decreasing Frequency of MedDRA Coded PT - Ph1b



**Preliminary data subject to change following final data cleaning efforts*

(P 184) PHASE 1B TRIAL OF CAMSIRUBICIN, A NOVEL DOXORUBICIN ANALOG, WITH CONCOMITANT PEGFILGRASTIM IN SUBJECTS WITH ADVANCED SOFT TISSUE SARCOMA TO IDENTIFY A NEW MTD/RP2D

Victoria Chua, MD, CLS[1]; Erlinda M. Gordon, MD[2]; Sant P. Chawla, MD, FRACP[1]; Holli Carlson, BS[3]; Chandler Robinson, MD[3]; Pierre Jordaan, MD[3]; Patrice Rioux, MD[3]; Lee D. Cranmer, MD, PhD, F.A.C.P.[4]; Aidan Kelly, BS[3], (1) Sarcoma Oncology Center, Santa Monica, CA, (2) Sarcoma Oncology Research Center, Santa Monica, CA, (3) Monopar Therapeutics, Inc., Wilmette, IL, (4) University of Washington/Fred Hutchinson Cancer Center, Seattle, WA

Objective: Doxorubicin is the 1st line treatment for many patients with advanced soft tissue sarcoma (ASTS). The clinical benefit of doxorubicin is limited by having to terminate treatment once a lifetime cumulative dose is reached due to the increased risk of irreversible cardiotoxicity. Camsirubicin HCl (camsirubicin) has been engineered to modify doxorubicin side chains that have been implicated in mediating cardiotoxicity, while still retaining antitumor activity. The hypothesis is that camsirubicin can take advantage of the known dose-response of this class of drug and be administered without restrictions on cumulative dose due to avoidance or reduction in cardiotoxicity. The objective of this phase 1b study is to identify a new maximum tolerated dose (MTD)/RP2D when camsirubicin is administered with concomitant pegfilgrastim and to assess the safety and anti-tumor activity of this regimen.

Methods: Like most anthracyclines, camsirubicin exhibits the dose limiting toxicity of neutropenia as demonstrated in a previously completed phase 1 trial, which identified 265 mg/m2 every 21 days (Q21D) as the recommended phase 2 dose (RP2D). A single arm phase 2 study administered camsirubicin at the RP2D with concomitant pegfilgrastim to minimize neutropenia which allowed administration of camsirubicin for up to 16 cycles (with cumulative doses >5000 mg/m2) with no evidence of irreversible cardiotoxicity. Given the lack of toxicity at the RP2D administered with concomitant pegfilgrastim in that phase 2 study, the current phase 1b study has been designed to dose escalate beyond the original RP2D in the presence of pegfilgrastim.

Results: : Data will be presented describing the safety and antitumor activity of camsirubicin at dose levels greater than the 265 mg/m2 previously evaluated with concomitant pegfilgrastim. To date, the first five dose levels (ranging between 265 and 650 mg/m2) have been accrued. No dose-limiting toxicities have been observed, and camsirubicin has demonstrated very good tolerability and early signs of clinical benefit. The majority of patients have had stable disease, including all three at the 520mg/m2 dose level. One patient at the 520 mg/m2 dose level, with

unresectable cancer at study entry, was deemed eligible for tumor resection after 6 cycles of camsirubicin treatment and a corresponding 21% reduction in tumor dimensions. This patient underwent successful surgical resection of the cancer with clear margins. No subjects to date have demonstrated signs or symptoms of drug-related cardiac dysfunction. This trial continues to show less frequent and less severe alopecia and oral mucositis with camsirubicin compared to what has been seen in recent ASTS clinical trials with doxorubicin. We anticipate availability of subject data for additional dose levels at the time of the conference, as the trial is still in progress at the time of this abstract's submission.

Conclusion: : Camsirubicin, a novel doxorubicin analog, when given with concomitant pegfilgrastim can be given at doses higher than the previously reported RP2D. The objective of this ongoing study is to continue to increase the dose and define a new MTD/RP2D in the presence of pegfilgrastim. The hypothesis is that higher per cycle and cumulative doses of camsirubicin will lead to an increase in tumor response and clinical benefit when compared to doxorubicin, where the cumulative dose is restricted. The identification of a new RP2D for camsirubicin will allow direct comparison to doxorubicin in subjects with ASTS as well as other cancer types where doxorubicin is currently used.

(P 185) LONGITUDINAL MUTATIONAL ANALYSIS OF TP53 IN CIRCULATING TUMOR DNA IN THE PLASMA OF PATIENTS WITH LIPOSARCOMA IN A PHASE I STUDY OF BRIGIMADLIN (BI 907828), AN MDM2-P53 ANTAGONIST

Patrick Schöffski, MD, MPH[1]; Michael Teufel, PhD[2]; Alexander Peltzer, MD[3]; Raphael Hesse, MD[3]; Patricia LoRusso, MD[4]; Noboru Yamamoto, MD[5]; Sant P. Chawla, MD, FRACP[6]; Peter Reichardt, MD, PhD[7]; Girish Jayadeva, MD[2]; Mehdi Lahmar, MD[8]; Mrinal Gounder, MD[9], (1) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium, (2) Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT, (3) Boehringer Ingelheim Pharma GmbH & Co. KG, Department of Translational Medicine & Clinical Pharmacology, Biberach an der Riss, Baden-Wurttemberg, Germany, (4) Yale University School of Medicine, Yale Cancer Center, New Haven, CT, (5) Department of Experimental Therapeutics, National Cancer Center Hospital, Tokyo, Tokyo, Japan, (6) Sarcoma Oncology Center, Santa Monica, CA, (7) Helios Klinikum Berlin-Buch and Medical School Berlin, Berlin, Berlin, Germany, (8) Boehringer Ingelheim International GmbH, mainz, Rheinland-Pfalz, Germany, (9) Memorial Sloan Kettering Cancer Center, New York, NY

Objective: The TP53 gene is the most frequently mutated gene in human cancer, is defined as a tumor suppressor gene and its gene product p53 plays a crucial role in preventing cancer formation. Inactivation of p53 can occur due to TP53 mutations or downregulation of wild-type p53 by its primary negative regulator, MDM2. Targeting the MDM2–p53 interaction can restore p53 function. Brigimadlin (BI 907828), a highly potent, orally available MDM2–p53 antagonist, is being evaluated in an ongoing Phase Ia/b study (NCT03449381) in patients with advanced solid tumors, including dedifferentiated liposarcoma (DDLPS). Brigimadlin has demonstrated early signs of preliminary efficacy and a manageable safety profile (LoRusso et al., Cancer Discovery 2023; LoRusso et al., ASCO 2023). Mutations in TP53 are considered as a potential mechanism of resistance to MDM2 antagonists. Here we present data from a longitudinal mutational analysis of TP53 using circulating tumor DNA (ctDNA), with the objective to assess if mutations in TP53 are associated with possible acquired resistance to brigimadlin.

Methods: Collection of blood samples for ctDNA analyses was optional in Phase Ia and mandatory in Phase Ib; samples were taken longitudinally from patients at baseline and every cycle until the time of the last available sample (LAS). ctDNA was purified from plasma and analyzed using tumor-specific next generation sequencing (NGS; custom-made 8-gene panel including TP53 using KAPA HyperCap technology) to identify tumor-derived somatic mutations that may be relevant to identify potential resistance mechanisms to brigimadlin. The limit of detection was determined to be 0.5% mutant allele frequency; common polymorphisms were filtered out.

Results: At data cut-off (March 28, 2023), 69 patients with DDLPS had been enrolled to receive brigimadlin Q3W, of whom 65 were evaluable for response according to RECIST. Plasma samples for ctDNA analysis were available from 21/65 (32%) patients. Baseline samples from 16 (25%) and the LAS from 17 (26%) patients were analyzed. The median interval between baseline sampling and LAS was 128 days (range, 43–631). As expected, the vast majority of patients (15/16; 94%) had no detectable mutations in TP53 at baseline; the one aberration detected (K373fs) is not likely to be a loss-of-function (LoF) mutation. At LAS, no (0) mutations were found in 7 (40%) samples, 1 mutation in 4 (24%), 2 mutations in 4 (24%), 5 mutations in 1 (6%), and 6 mutations in 1 sample (6%). No sample had more than 6 mutations. The most frequent mutations found were R175H, R273H, R248Q/W and R280G (across 5 of 33 samples analyzed [15%]), all of which are known TP53 hotspot mutations and are LoF. No clear association of induction of mutations could be identified for any brigimadlin dose, dosing schedule, or treatment duration. Only 7/17 (41%) patients still showed wild-type TP53 status at LAS. Signs of efficacy were observed in patients with acquired TP53 LoF mutations, suggesting that these have no effect on efficacy. Analysis of TP53 mutational status at LAS in conjunction with correlative clinical efficacy will be presented.

Conclusion: This mutation analysis represents one of the most robust assessments of longitudinal ctDNA by NGS for TP53 from an MDM2–p53 antagonist trial in liposarcoma. While acquired mutations in TP53 are very common, these data suggest that brigimadlin does not systematically lead to broad acquisition of resistance by inducing alterations in TP53.

(P 186) PATIENT REPORTED OUTCOME ASSESSMENT AND REPORTING IN RANDOMIZED CLINICAL TRIALS OF SYSTEMIC THERAPY FOR ADVANCED SOFT TISSUE SARCOMAS: A SYSTEMATIC REVIEW.

Evelyne Roets, MD[1]; Winette van der Graaf, Prof. Dr.[2]; Bauke van Riet, MD[1]; Eugenie Younger, MD[3]; Francesco Sparano, MSc[4]; Roger Wilson, CBE HonMD HonLLD[5]; Stevie van der Mierden, MSc[1]; Neeltje Steeghs, Dr.[2]; Fabio Efficace, MD PhD[4]; Olga Husson, PhD[1], (1) Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands, (2) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (3) Royal Marsden NHS Foundation Trust, London, England, United Kingdom, (4) Health Outcomes Research Unit, Italian Group for Adult Hematologic Diseases (GIMEMA) Data Center, Rome, Lazio, Italy, (5) Founder - Sarcoma UK, London, England, United Kingdom

Objective: In advanced soft tissue sarcoma (STS), improvement of health-related quality of life (HRQoL) is a primary goal of treatment. The primary objective of this systematic review is to assess to what extent patient reported outcomes (PROs) are reported as a primary, secondary or exploratory outcome within randomized controlled trials (RCTs) of systemic treatments for STS patients with advanced disease. Secondary objectives are to describe the type of PROs

measured (e.g. HRQoL), how often PROs are reported in the primary or secondary publication and, if applicable, the duration between primary and secondary publication, tools used to collect PRO data and the quality of reporting of PRO data.

Methods: A systematic literature search from January 2000 – August 2022 was conducted for phase II/III RCTs evaluating systemic treatments in adult patients with advanced STS. RCTs with less than 50 patients, gastrointestinal stromal tumors or HIV-associated Kaposi sarcoma were excluded. Data was captured through electronic data extraction forms by two independent researchers. A third reviewer was consulted in case of any discrepancies. The quality of PRO reporting was assessed according to the CONSORT (Consolidated Standards of Reporting Trials) PRO criteria extension. The number of RCTs that adhered to each CONSORT PRO item, as well as the total CONSORT PRO adherence score for each RCT were reported.

Results: The literature search resulted in 7294 records of which 59 articles, comprising 43 RCTs, were included (Fig. 1). Of these, only 10 RCTs (23%) also included PROs, as either secondary or exploratory outcomes in 8 and 2 RCTs, respectively. In nearly all RCTs (9/10) PROs were assessed with the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core-30 (EORTC QLQ-C30), in some cases in conjunction with another PRO instrument such as the EQ visual analogue scale (EQ-VAS) (1/10), fatigue-specific FA-13 questionnaires (1/10), the EQ-5D questionnaire (2/10) and the modified Brief Pain inventory-short form (mBPI-sf) (1/10). PROs were reported in the primary (6/10) or secondary publication (1/10) or in both the primary and secondary publication (3/10). The median time between primary and secondary publication was 23 months (range 12-39 months). The median adherence score for the CONSORT-PRO extension was 5.5/14 (range 2/14 – 11/14) with the poorest reported items being: describing the PRO hypothesis and domains (2/10 and 0/10), mode of administration (1/10), strategy for dealing with missing data (4/10), reporting PRO study limitations (4/10), generalizability of results (3/10) and relation with clinical outcomes (4/10) (table 1). The adherence score was higher in publications focusing exclusively on PROs (11/14, 10/14, 11/14 and 11/14).

Conclusion: Although HRQoL is a critical aspect in patients with advanced STS, most RCTs conducted thus far in these patients did not include HRQoL or other types of PROs, and in none of the RCTs identified, PROs were included as a primary endpoint. Despite development of the CONSORT-PRO extension in 2013, quality of PRO reporting in RCTs was extremely poor. Higher quality of PRO reporting is needed to more robustly inform patient care in the setting of advanced STS.

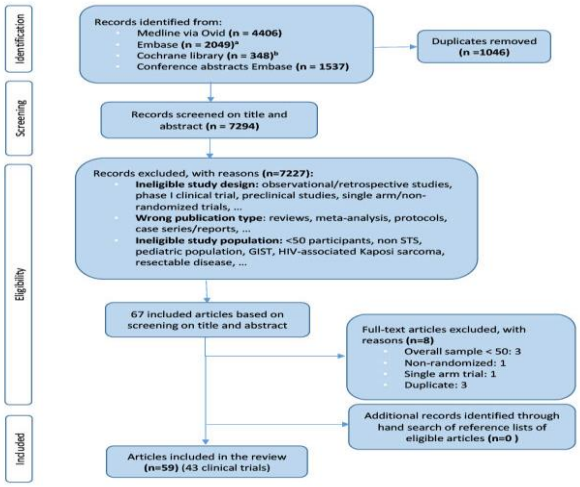


Fig. 1 Schematic breakdown of literature search. STS, soft tissue sarcoma.

[a] Without conference abstracts

[b] Results limited to sources: international Clinical Trials Registry Platform, ClinicalTrials.gov, CINAHL database

		Seddon et al. [1]	Van Tine et al. [2]	Younger et al. [3]	Hudgens et al. [4]	Somaiah et al. [5]	Coens et al. [6]	Grunwald et al. [7]	Schmoll et al. [8]	Gounder et al. [9]	Le Cesne et al. [10]	Total number of RCTs adhering to each item (n, %)
Item of CONSORT PRO extension	score											
PRO noted as primary/secondary endpoint in the abstract												
Yes	1				■				■	■		3 (30)
PRO mentioned but unclear endpoint status	0.5		■					■			■	3 (30)
Background and rationale for including PROs	1		■		■		■			■	■	5 (50)
PRO hypothesis present	0.5				■		■					2 (20)
PRO domains specified in hypothesis	0.5											0 (0)
Evidence of PRO instrument validity provided/cited[a]	1		■		■		■	■		■	■	6 (60)
Statement of the person completing the PRO measure (e.g. 'patients completed', or 'self-report')	0.5		■	■	■	■	■	■		■		7 (70)
Mode of administration specified (e.g. paper, e-PRO)	0.5							■				1 (10)
Statistical approach for dealing with missing data specified (e.g. imputation, omission of cases with missing data)	1		■				■	■		■		4 (40)
Report number of questionnaires submitted/available for analysis at baseline	0.5		■	■	■		■	■	■	■	■	8 (80)
Report number of questionnaires submitted/available for analysis principle timepoint for PRO analysis	0.5	■	■	■	■		■		■	■	■	8 (80)
Table including baseline PRO findings	1	■			■		■	■		■		5 (50)
Number of patients (denominator) included in each PRO analysis and whether this was intention to treat	1	■	■		■		■			■		5 (50)
PRO results reported for the hypothesized domains and time point specified in the hypothesis –OR- reported for each domain of the PROM if no PRO hypothesis provided	0.5	■	■		■		■	■				5 (50)
Results include confidence intervals, effect size or some other estimate of precision	0.5	■	■	■	■	■	■	■		■	■	9 (90)
Results of any subgroup/adjusted/exploratory analyses are reported	1		■		■	■	■			■		5 (50)
PRO study limitations provided	1		■	■			■			■		4 (40)
Implications of PRO results for generalizability, use in clinical practice	1		■	■	■						■	3 (30)
PROs interpreted in relation to clinical outcomes	1		■				■				■	4 (40)
Total CONSORT PRO score[b]	14	3.5	11	4	10	2	11	6	2	11	5	

Table 1 Quality of patient-reported outcomes (PRO) according to the CONSORT PRO extension

Abbreviations: PRO, Patient-reported outcome; CONSORT, Consolidated Standards of Reporting Trials. [a]Studies using multiple PRO instruments were also rated as "using PRO validated instruments" if at least one of the PRO instruments used was validated. [b] As none of the RCTs had PROs as a primary outcome and none of the RCTs used PROs for trial stratification the maximum achievable score for the checklist was 14.



(P 187) PALLIATIVE LATTICE STEREOTACTIC BODY RADIOTHERAPY FOR PATIENTS WITH SARCOMA

Michael R. Waters, MD PhD[1]; Sai Duriseti, MD[2]; Douglas Caruthers, MS[1]; Yi Huang, MS[1]; Jeff Szymansky, MD PhD[1]; Dinesh Thotola, PhD[1]; Angela Hirbe, MD,PHD[3]; Brian Andrew Van Tine, MD, PhD[4]; Mia C. Weiss, MD[4]; Jeff Michalski, MD[1]; Aadel Chaudhuri, MD PhD[1]; Clifford Robinson, MD[1]; Hyun Kim, MD[5]; Christopher Abraham, MD[1]; Shahed Badiyan, MD[1]; Sreekrishna Goddu, PhD[1]; Tom Mazur, PhD[1]; Nels Knutson, PhD[1]; Pamela Samson, MD[1]; Anthony J. Apicelli, III, MD, PhD[1]; Matthew Spraker, MD PhD[1], (1) Washington University in St Louis, St Louis, MO, (2) UCLA, Los Angeles, CA, (3) Washington University in St. Louis, St. Louis, MO, (4) Washington University School of Medicine, St. Louis, MO, (5) Washington University in St Louis, St. Louis, MO

Objective: Metastatic or unresectable disease is the cause of cancer-related morbidity and mortality for approximately 90% of cancer patients. Recent data demonstrates that stereotactic body radiation therapy (SBRT) offers improvements over conventionally fractionated radiotherapy for palliative treatment. SBRT, however, may be associated with increased toxicity and decreased control in larger tumors. Spatially fractionated radiation therapy (SFRT) may address these limitations of traditional SBRT. Unlike conventional radiotherapy, SFRT creates a heterogeneous dose field with high-dose “peaks,” organized throughout a target volume with intervening low-dose “valleys”. Lattice radiotherapy is a form of SFRT that uses a 3-dimensional beam arrangement to target high-dose spherical volumes within the global target. In this prospective trial (NCT04553471) the authors describe the effectiveness of lattice SBRT in local control of large, unresectable lesions with palliative intent.

Methods: Patients >18 years old, ECOG performance status ≤ 2, with histologically or cytologically confirmed sarcoma (including extremity), thoracic cancer, abdominal cancer, or pelvic cancer planning to undergo palliative radiotherapy to a lesion ≥ 4.5 cm were eligible for the Lattice radiation therapy. Exclusion criteria included prior overlapping high-dose radiation therapy, patients with tumors in need of urgent surgical intervention, and those receiving any cytotoxic cancer therapy regimens. Eligible patients underwent Lattice SBRT prescribed to a dose of 20 Gy in 5 fractions with a simultaneous integrated boost of 66.7 Gy in 5 fractions, delivered every other day. The co-primary endpoints of the study were rate of local control of the treated (i.e. target) lesions at 180 days as determined by RECIST v1.1 criteria, and proportion of patients with treatment related grade 3+ CTCAE v5.0 toxicity.

Results: Seventy-seven patients were enrolled in this phase II study. Overall median survival for all patients was 11.5 months, and 13.8 months for 35 sarcoma patients. Median overall progression free survival was 6.8 months for all patients and 8.5 months for sarcoma patients. As local control was greater than 50% for both groups, median time to local failure could not be calculated. In sarcoma patients, at 180 days 88% (16/18) of patients displayed local control of disease by RECIST scoring (66% (12/18) stable disease, 17% (3/18) partial response, and 5% (1/18) complete response. Similarly, for patients with other cancer types at 180 days, 90% (29/32) of patients experienced local control of the target lesions (65% (21/32) stable disease, 21% (7/32) partial response, 3% (1/32) complete response). The therapy was well tolerated with 5 grade 2 toxicities reported, 1 grade 3 toxicity, and 1 grade 5 toxicity (a case of tumor lysis syndrome) possibly, probably, or definitely attributable to Lattice SBRT.

Conclusion: This phase II study demonstrates that Lattice SBRT is a promising treatment option for palliative care in patients with large tumors (≥ 4.5 cm). The trial recorded an impressive local control rate of 90% at 180 days for all patients evaluated by RECIST criteria. Additionally noteworthy was Lattice SBRT's efficacy in patients with sarcoma with a local control rate of 88% at 180 days. The treatment was generally well-tolerated with minimal severe toxicities reported, suggesting it is a viable treatment option for patients with large tumors. The association of one instance of an attributable grade 5 toxicity (tumor lysis syndrome) with Lattice RT warrants careful consideration and further investigation. Given these encouraging results however, Lattice SBRT in the palliative setting is a safe and effective treatment for patients with large, unresectable tumors.

(P 188) IMPACT OF NIROGACESTAT ON FUNCTIONAL STATUS IN PATIENTS WITH DESMOID TUMORS: RESULTS FROM THE PHASE 3 DEFI STUDY

Bernd Kasper, MD, PhD[1]; Mrinal Gounder, MD[2]; Ravin Ratan, MD[3]; Timothy Bell, MHA[4]; Cristina Ivanescu, PhD[5]; James Marcus, PhD[6]; Allison Lim, PharmD[7]; Sandra Goble, MS[7]; Sunny Cho, PharmD[7]; Thierry Alcindor, MS, MSc[8]; Winette van der Graaf, Prof. Dr.[9]; Patrick Schöffski, MD, MPH[10]; Breelyn A. Wilky, MD[11]; charlotte Benson, MBChB, FRCP[12]; Nam Bui, MD[13]; Rashmi Chugh, MD[14]; Shivaani Kummar, MD, FACP[15]; Richard F. Riedel, MD[16], (1) University of Heidelberg, Mannheim University Medical Center, Mannheim, Baden-Wurttemberg, Germany, (2) Memorial Sloan Kettering Cancer Center, New York, NY, (3) The University of Texas MD Anderson Cancer Center, Houston, TX, (4) SpringWorks Therapeutics, Inc., Stamford, CT, (5) IQVIA, Amsterdam, Noord-Holland, Netherlands, (6) IQVIA, Washington, DC, (7) SpringWorks Therapeutics Inc, Stamford, CT, (8) McGill University, Montreal, PQ, Canada, (9) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (10) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium, (11) University of Colorado Cancer Center, Aurora, CO, (12) The Royal Marsden Hospital, London, England, United Kingdom, (13) Stanford University, Stanford, CA, (14) University of Michigan Rogel Cancer Center, Ann Arbor, MI, (15) Oregon Health & Science University, Portland, OR, (16) Duke Cancer Institute, Durham, NC

Objective: Patients with desmoid tumors (aggressive fibromatosis) can experience severe pain and functional impairment (e.g., difficulty walking, using the toilet, and lifting heavy objects); in addition, symptoms can negatively affect work, school, and relationships with friends or family. In the phase 3 DeFi trial of adult patients with progressing desmoid tumors, nirogacestat (n=70) demonstrated statistically significant and clinically meaningful improvement in the primary endpoint of progression-free survival (HR: 0.29 [95% CI, 0.15–0.55]; p< 0.001) and in all key secondary efficacy endpoints (p < 0.01) compared with placebo (n=72). Secondary and exploratory DeFi endpoints included different aspects of patient-reported functional status to further characterize nirogacestat's treatment effect.

Methods: In DeFi, patients completed prespecified functional status assessment tools at baseline and at each 28-day cycle during the first year of treatment: the GOunder/Desmoid Tumor Research Foundation DEsmoid Impact Scale (GODDESS-DTIS) Physical Functioning domain (moving, reaching, vigorous activity, moderate activity, accomplishing less; 7-day recall); the European Organisation for Research and Treatment of Cancer Core Quality of Life Questionnaire (QLQ-C30) Physical Functioning (strenuous activities, taking a long walk, taking a short walk, need to stay in a bed or chair, help with eating/dressing/washing/using the toilet; 7-day recall) and Role Functioning (work/other daily activities, hobbies/leisure activities; 7-day recall) domains; and the Patient-Reported Outcomes Measurement Information System Physical Function Short Form 10a (PROMIS PF10a; dexterity/upper extremities, walking/mobility/lower extremities, neck/back, daily activities such as errands; 7-day recall). Changes from baseline in functional status scores were compared between nirogacestat and placebo at cycle 10 using a mixed model with repeated measures (MMRM). Cycle 10 was preselected to allow adequate time for a treatment effect to be observed.

Results: Statistically significant improvements from baseline in physical and role functioning were observed with nirogacestat compared with placebo at cycle 10 across all assessment tools (Table). Differences between the nirogacestat and placebo groups emerged as early as cycle 3 for some aspects of patient-reported functional status.

Conclusion: Significantly greater improvement in different aspects of functional status was observed with nirogacestat compared with placebo across multiple assessment tools in adult patients with desmoid tumors. Because patients with desmoid tumors can experience substantial functional impairment, improvement of functional status is an important treatment goal.

Table. Changes from baseline in physical and role functioning assessment scores at cycle 10

Assessment	Change from baseline at cycle 10				Difference between treatment arms	
	Nirogacestat		Placebo			
	n	LS mean (SE)*	n	LS mean (SE)*	Value	One-sided p-value*
GODDESS-DTIS Physical Functioning	39	−0.6 (0.10)	28	0.1 (0.10)	−0.7 (0.14)	<0.001
QLQ-C30 Physical Functioning	38	9.1 (1.77)	28	−5.2 (1.93)	14.4 (2.54)	<0.001
QLQ-C30 Role Functioning	38	13.3 (3.13)	28	−5.6 (3.39)	18.9 (4.51)	<0.001
PROMIS Physical Functioning (PF10a)	39	5.5 (0.79)	28	−0.5 (0.83)	6.0 (1.10)	<0.001

Assessment scale ranges (and direction of improvement): DTIS PF, 1–5 (↓); QLQ-C30 PF and RF, 0–100 (↑); PROMIS PF10a T-score, 13.5–61.9 (↑).

*LS mean differences, SE, and p-values were calculated using a mixed model with repeated measures (MMRM) with treatment and visit as factors, corresponding baseline score and primary tumor location (intra-abdominal or extra-abdominal) as covariates, and included baseline by visit and treatment by visit interactions.

LS, least squares; PF, Physical Functioning; RF, Role Functioning; SE, standard error.

(P 189) INTERNATIONAL RETROSPECTIVE STUDY FROM THE ULTRA-RARE SARCOMA WORKING GROUP ON LOW-GRADE FIBROMYXOID SARCOMA AND SCLEROSING EPITHELIOID FIBROSARCOMA: OUTCOME OF ADVANCED DISEASE AND SYSTEMIC THERAPIES

Claudia Giani, MD[1]; Silva Ljevar, MSc[2]; Alexander J. Lazar, MD, PhD[3]; Andrea Napolitano, MD[4]; Elizabeth Connolly, MD[5]; Emanuela Palmerini, MD, PhD[6]; Roberto Scanferla, MD[7]; Jyoti Bajpai, MD, DM[8]; Daniel Wong, n/a[9]; Koichi Ogura, MD, PhD[10]; Chia-Chen Li, MD[11]; Jonathan Trent, MD, PhD[12]; Alexander Lee, Consultant[13]; Yeh Chen Lee, MBBS BMedSci MPhil FRACP[14]; Susie Bae, MBBS, MSc[15]; Sosipatros A. Boikos, MD[16]; Hanna Koseła-Paterczyk, MD PhD[17]; Antonella Brunello, MD, PhD[18]; Giacomo Giulio Baldi, MD[19]; Herbert Ho Fung Loong, MBBS(HK), PDipMDPath(HK), MRCP(UK), FRCP Edin, FHKCP, FHKAM(Medicine)[20]; Fernando Campos, MD[21]; Robert G. Maki, MD PhD[22]; Anna Maria Frezza, MD[23]; Lorenzo D'Ambrosio, MD[24]; Andrew J. Wagner, MD, PhD[25]; Marta Sbaraglia, MD[26]; Inga-Marie Schaefer, MD[27]; Alessandro Gronchi, MD, FSSO[28]; Rosalba Miceli, MD, PhD[2]; Silvia Stacchiotti, MD[23], (1) Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (2) Unit of Biostatistics for Clinical Research Department of Epidemiology and Data Science, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (3) The University of Texas MD Anderson Cancer Center, Houston, TX, (4) The Royal Marsden NHS, London, UK, London, England, United Kingdom, (5) Department of Medical Oncology, Chris O'Brien Lifehouse, Sydney, Australia. Faculty of medicine and health, University of Sydney, Sydney, Australia., Sydney, New South Wales, Australia, (6) IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (7) Orthopedics Oncology and Reconstructive Surgery Unit, Azienda Ospedaliero Universitaria Careggi, Florence, Italy, Firenze, Toscana, Italy, (8) Tata Memorial Centre,Homibhabha National University,Mumbai,India, Mumbai, Maharashtra, India, (9) Sir Charles Gairdner Hospital (SCGH), Australia, Nedlands, Western Australia, Australia, (10) National Cancer Center

Hospital, Tokyo, Tokyo, Japan, (11) National Taiwan University Cancer Center, Taipei, Taipei, Taiwan (Republic of China), (12) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, (13) The Christie NHS Foundation Trust, Manchester, England, United Kingdom, (14) Prince of Wales Hospital, Randwick, New South Wales, Australia, (15) Peter MacCallum Cancer Centre, Melbourne, VIC, Australia, Melbourne, Victoria, Australia, (16) Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, (17) Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (18) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy, Padua, Veneto, Italy, (19) Medical Oncology Department, "Santo Stefano" Hospital, Prato, Italy, Prato, Toscana, Italy, (20) The Chinese University of Hong Kong, Hong Kong, N/A, Hong Kong, (21) Medical Oncology Department, A.C.Camargo Cancer Center, São Paulo, SP, Brazil, Sao Paulo, Sao Paulo, Brazil, (22) Memorial Sloan Kettering Cancer Center; Professor, Weill Cornell Medical College, New York, NY, (23) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (24) University if Turin, Turin, Italy, Torino, Piemonte, Italy, (25) Dana-Farber Cancer Institute, Boston, MA, (26) Department of Pathology, Azienda Ospedale-Università Padova, Padova, Italy, Padova, Veneto, Italy, (27) Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA, Boston, MA, (28) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy

Objective: To report on the outcome of pts with metastatic low-grade fibromyxoid sarcoma (F) and sclerosing epitheliod sarcoma (S).

Methods: We conducted an international retrospective multicentre study of all consecutive patients (pts) affected by metastatic F and S, observed and treated at the participating institutions of the Utra-Rare Sarcoma Working Group.
Eligible pts had a pathologic diagnosis of F, S or hybrid F/S (requiring strong MUC4 expression and/or the presence of one of the following: FUS/EWSR1 rearrangement, EWSR1/FUS::CREB3L1/CREB3L2/CREM, YAP1::KMT2A). Hybrid F/S were included in the S group. Primary end-point was progression-free survival (PFS), defined as the time from diagnosis of distant metastases until first progression or death, whichever occurred first. Secondary end-points were overall response rate (ORR), and post-metastasis overall survival (OS), defined as the time from the onset of the first distant metastasis and death or last follow-up (FU). PFS and OS were estimated with the Kaplan-Meier method, overall and for the two F and S groups.
A propensity score (PS) matching analysis (variables: age, sex, histology, stage at diagnosis, primary site, surgery) of pts treated vs not treated with first-line was done and weighted Cox models for OS and PFS were fitted to estimate the treatment effect.
The analysis presented herein was performed on cases entered in the database by June 10th 2023; data collection is still ongoing (closure planned by August 31st 2023) and the updated analysis will be presented at the meeting.

Results: 303 eligible pts were identified, 86/303 (28.4%) were metastatic (25 F, 61 S) and are the subject of this analysis (Table 1). 18 F and 49 S pts received at least one line of systemic therapy (Rx) (Table 2).
At a 62.14-mo m-FU (IQR:31.1-82.5), m-OS was: overall = 58.4 (IQR:32.4-NA) mos, F = not reached (IQR:32.4-NA), S = 58.4 (IQR:30.5-83.2) mos; m-PFS was: overall = 23.3 (IQR:9.0-56.4) mos, F = 24.5 (IQR:14.3-67.5) mos, S = 20.3 (IQR:8.8-56.4) mos. Median time to DM was: overall = 29 (IQR:16.7-85.7) mos, F = 79.2 (IQR:62.9-82.3) mos, S = 18 (IQR:17.3-98.8) mos.

In the anthracycline-based Rx group, 1/12 response (PR) was seen in F (ORR 7.1%), 0/29 in S.

At 58.9-mo m-FU (IQR:22.6-69.01), m-OS and m-PFS were: overall = 30.5 (IQR:16.4-51.0) and 5.1 (IQR:2.0-20.1) mos, F = 30.6 (IQR:12.6-NA) and 2.6 (IQR:2.0-12.6) mos, S = 29.9 (IQR:23.9-51.0) and 6.8 (IQR:2.0-23.9) mos.
In the gemcitabine-based Rx group, 1/7 PR was seen in F (ORR 14.3%), 0/17 in S. At 81.7-mo m-FU (IQR:31.9-118.0), m-OS and m-PFS were: overall = 69.3 (IQR:32.4-NA) and 29.2 (IQR:8.1-60.3) mos, F = NA (IQR:NA-NA) and 32.4 (IQR:17.3-81.3) mos, S = 48.9 (IQR:30.5-80.1) and 24.2 (IQR:7.5-60.3) mos.
In the pazopanib group, 2/6 PR were seen in F (ORR 28.6%), 1/19 in S (ORR 4.3%). At 36.5-mo m-FU (IQR:13.7-66.2), m-OS and m-PFS were: overall = 29.3 (IQR:13.4-NA) and 10.7 (IQR:2.8-19.5) mos, F = NA (IQR:13.4-NA) and 13.4 (IQR:2.0-19.5) mos, S = 24.4 (IQR:12.0-NA) and 10.6 (IQR:2.8-18.1) mos.
No responses were seen to trabectedin. At 39.7-mo m-FU (IQR:19.6-43.4), m-OS and m-PFS were: overall = 15.0 (IQR:8.4-41.3) and 3.3 (IQR:2.0-7.4) mos, F = NA (IQR:8.4-NA) and 6.2 (IQR:2.3-11.0) mos, S = 14.5 (IQR:8.3-20.5) and 2.1 (IQR:1.9-6.2) mos.
In the “other” Rx group, activity was seen to ifosfamide and oral cyclophosphamide.

The comparison of treated vs not treated pts with first line Rx did not result in a statistically significant difference in PS-adjusted PFS (HR 0.88, 95%CI 0.4-1.94, p-value: 0.759).

Conclusion: The metastatic course of F and S may be indolent. When systemic therapy is needed, gemcitabine-based regimen and pazopanib currently seem to be most active. Responses were seen also to ifosfamide and oral cyclophosphamide. New, effective systemic agents are needed.

Table 1. Patients characteristics.

Eligible pts (LGFMS - SEF)	303 (215 - 88)	
Total number of metastatic pts (%)	86/303 (28.4)	
Pts metastatic at diagnosis (%)	41/303 (13.5)	
Pts metastatic at relapse (%)	45/303 (14.9)	
Hystology	**LGFMS**	**SEF**
Number of pts with metastatic disease (%)	25/215 (11.6)	61/88 (69.3)
Metastatic at diagnosis (%) Metastatic at relapse (%)	17/215 (7.9) 8/215 (3.7)	24/88 (27.3) 37/88 (42.0)
Age at diagnosis (years), median (IQR) Male/Female (%)	45 (37-54) 13 (52.0)/12 (48.0)	43 (34-57) 35 (57.4)/26 (42.6)
Hystopathological features		
MUC4 expression (%) *Yes* *No* *Unknown*	 15 (60.0) 0 (0.0) 10 (40.0)	 44 (72.1) 1 (1.6) 16 (26.2)
FUS rearrangement (%) *Positive* *FUS::CREB3L2* *FUS::CREB3L1* *EWSR1::CREB3L1* *Other* *Negative* *Not done*	 15 (60.0) 8/15 (53.3) 0/15 (0.0) 3/15 (20.0) 4/15 (26.7) 2 (8.0) 8 (32.0)	 - - - - - - -
EWSR1::CREB3L1 **(%)** *Positive* *Negative* *Not done* *Other rearrangements* ***	 - - - -	 24 (39.3) 14 (23.0) 23 (35.7) 11/14 (78.6)
Site		
Primary site (%) *Extremities* *Abdomen/retroperitoneum* *Chest wall/back/paraspinal region* *Other*	 10 (40.0) 7 (28.0) 4 (16.0) 4 (16.0)	 20 (32.8) 21 (34.4) 10 (16.4) 10 (16.4)
Site of metastases (%) **Single site** *Lung* *Bone* *Soft tissues* *Liver* *Other* **Multiple sites**	 13 (52.0) 5/13 (38.5) 0/13 (0.0) 5/13 (38.5) 1/13 (7.7) 2/13 (15.3) 12 (48.0)	 36 (59.0) 19/36 (52.8) 6/36 (16.7) 2/36 (5.5) 0/36 (0.0) 9/36 (25.0) 25 (41.0)
Treatment of metastatic disease		
Surgery for metastatic site (%) **No** **Yes**	 17 (68.0) 8 (32.0)	 36 (59.1) 25 (40.9)

Table 2. Systemic treatments.

	LGFMS	SEF
Total number of pts treated with chemo (%)	18/25 (72.0)	49/61 (80.3)
Anthracycline-based regimens (%)		
Number of pts (%)	14 (77.7)	32 (65.3)
Monotherapy	5/14 (35.7)	9/32 (28.1)
+ ifosfamide	6/14 (42.9)	17/32 (53.1)
+ other agents	3/14 (21.4)	6/32 (18.8)
Number of cycles, median (IQR)	3 (2-6)	5 (2-6)
Line of treatment (%)		
First	13 (92.9)	24 (75.0)
Second	1 (7.1)	6 (18.8)
Third or subsequent	0 (0.0)	2 (6.2)
Pts evaluable for response (%)	12 (85.7)	29 (90.6)
Best response		
CR	0	0
PR	1	0
SD	4	16
PD	7	13
ORR (%)	7.1	0.0
Gemcitabine (%)		
Number of pts (%)	7 (38.9)	19 (38.8)
Monotherapy	3/7 (42.9)	2/19 (10,5)
+ taxane	4/7 (57.1)	17/19 (89.5)
Number of cycles, median (IQR)	6 (4.5-9)	3 (2-5)
Line of treatment (%)		
First	2	5
Second	2	8
Third or subsequent	3	4
NA	0	2
Pts evaluable for response (%)	6 (85.7)	17 (89.5)
Best response		
CR	0	0
PR	1	0
SD	4	8
PD	2	9
ORR (%)	14.3	0.0
Pazopanib (%)		
Number of pts (%)	7 (38.9)	23 (46.9)
Number of cycles, median (IQR)	2 (1.5-2.5)	3.5 (2.75-7)
Line of treatment (%)		
First	2 (28.6)	7 (30.4)
Second	3 (42.9)	7 (30.4)
Third or subsequent	0 (0.0)	8 (34.8)
NA	2 (28.6)	1 (4.3)

(P 190) DOES RESPONSE TO PRIOR CHEMOTHERAPY PREDICT OUTCOME FROM FURTHER SYSTEMIC TREATMENT IN ADVANCED LEIOMYOSARCOMA? A SINGLE UK CENTRE EXPERIENCE

Alexander Lee, Consultant[1]; Hassan Hameed, MD[1]; Ann Buchan, Sarcoma Specialist Nurse[1]; Charlotte L. Young, Specialist Nurse[1]; Madeleine C. Strach, MD[2]; Michael Leahy, MD PhD[1], (1) The Christie NHS Foundation Trust, Manchester, England, United Kingdom, (2) Chris O'Brien LifeHouse, Sydney, New South Wales, Australia

Objective: Leiomyosarcoma is an aggressive soft tissue sarcoma subtype. The development of unresectable and/or metastatic disease is typically rapidly fatal. Cytotoxic chemotherapies remain the mainstay of palliative treatment but are associated with limited efficacy and significant toxicity, with the potential for adverse quality of life. The means for identifying patients most likely to gain benefit from consecutive lines of palliative chemotherapy are limited. We undertook to assess whether response to prior chemotherapy could be a useful factor in selecting patients most likely to benefit from further lines of systemic treatment.

Methods: We performed a retrospective review of outcomes for all patients treated with 2 or more lines of palliative systemic anti-cancer therapy (SACT) (excluding anti-oestrogen agents) for advanced leiomyosarcoma at a single UK sarcoma centre between 2004 and 2022. Eligible patients were identified from a prospectively maintained departmental database that included contemporaneous data on chemotherapy response and progression-free intervals. Baseline patient and tumour characteristics were recorded, as were outcomes for each line of palliative SACT. Comparison of progression-free survival following 1st (CT1) and 2nd line (CT2) SACT was performed, with analysis of PFS following CT2 stratified by response and 12 week progression-free state (12wPFS) after CT1. We identified 61 patients who received at least 2 lines of palliative SACT for advanced leiomyosarcoma. 56/61 (92%) of patients were female.

Average age at start of CT1 was 53.0 years (range 31.5-75.3). Primary leiomyosarcoma site was uterus in 48%, retroperitoneal 25%, abdominopelvic 15%, extremity 10% and unknown 3%. Tumour grade per FNLCC was grade 1,2 and 3 in 3%, 31% and 66% respectively. Organs involved by metastatic disease were lung 61%, liver 33%, peritoneum 13%, bone 15% and soft tissue 8%. ECOG performance status at start of CT1 was 0, 1, 2, 3 and not recorded in 31%, 54%, 5%, 2% and 8%.

Results: CT1 regimen was doxorubicin-based in 90% (85% monotherapy, 5% combination with ifosfamide), gemcitabine-docetaxel 8%, trabectedin 2%. Median PFS for CT1 was 142 days (95% CI 74-205). Best response to CT1 was partial response (PR) in 9/61 (15%), stable disease (SD) 24/61 (39%) and disease progression (PD) 25/61 (41%). Best response to CT1 was not evaluable in 3/61 (5%). CT2 regimen was gemcitabine-docetaxel in 54%, ifosfamide 23%, doxorubicin 8%, trabectedin 8%, pazopanib 5% and carboplatin-paclitaxel in 2%. Median PFS for CT2 was 118.5 days (95% CI 70-169). Best response to CT2 was PR 10/61 (16%), stable disease 21/61 (34%) and disease progression 27/61 (34%). Best response to CT1 was not evaluable in 3/61 (5%). There was no significant correlation observed between matched CT1 PFS and CT2 PFS (Pearson R = 0.02). When stratified by best response to CT1 (PR/SD vs PD), there was no significant difference in CT2 PFS (median PFS 140.5 days [95%CI 84-178) vs 76 days [95%CI 64-189]). Similarly, when stratified by CT1 12wPFS there was no significant difference in CT2 PFS between those who achieved CT1 12wPFS or not (median PFS 108 days [95%CI 63-169] v 122 days [95%CI 64-226]). 37/61 (61%) of patients had further lines of SACT (3rd-5th line). These regimens were trabectedin in 32/61 (52%), gemcitabine-docetaxel 8/61 (13%), pazopanib/axitinib/anlotinib 8/61 (13%), ifosfamide 1/61 (2%). Efficacy outcomes for specific chemotherapy regimens regardless of line of treatment were as follows: - doxorubicin-based (n=60): median PFS 142 days; best response - PR 15%, SD 42%, PD 42%, NE 2% - gemcitabine-docetaxel (n=45): median PFS 157 days; best response – PR 22%, SD, 30%, PD 41%, NE 7% - trabectedin (n=38): median PFS 101 days: best response – PR 8%, SD 39%, PD 47%, NE 5% - ifosfamide (n=15): median PFS 70 days: best response – PR 13%, SD 27%, PD 60%

Conclusion: We found limited efficacy of palliative SACT in advanced leiomyosarcoma in our single centre cohort, with results consistent with the available published evidence base. Doxorubicin-based regimens and gemcitabine-docetaxel had similar efficacy outcomes in this series. Response and degree of benefit from 1st line SACT did not appear to predict for benefit from 2nd line SACT. These findings indicate that lack of benefit from first line chemotherapy should not be seen to disqualify patients with advanced leiomyosarcoma from proceeding with 2nd line treatment.

(P 191) **SARC024 COHORT D: PHASE 2 TRIAL OF REGORAFENIB IN CHILDREN, ADOLESCENTS, AND ADULTS WITH RHABDOMYOSARCOMA**

Leo Mascarenhas, MD, MS[1]; Rachel Heise, MS[2]; Lara Davis, MD[3]; Vicki L. Keedy, MD, MSCI, FASCO[4]; Steven Attia, D.O.[5]; Kristen N. Ganjoo, MD[6]; Scott Okuno, M.D.[7]; Karla V. Ballman, Ph.D.[2]; Robert G. Maki, MD PhD[8], (1) Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, (2) Weil Cornell Graduate School of Medical Sciences, New York, NY, (3) OHSU Knight Cancer Institute, Portland, OR, (4) Vanderbilt University Medical Center, Nashville, TN, (5) Mayo Clinic, Jacksonville, FL, (6) Stanford Cancer Center, Palo Alto, CA, (7) Mayo Clinic, Rochester, MN, (8) Memorial Sloan Kettering Cancer Center; Professor, Weill Cornell Medical College, New York, NY

Objective: Regorafenib is a multi-tyrosine kinase inhibitor that has shown clinical efficacy in several cancer types including some sarcomas like gastrointestinal stromal tumors, osteosarcoma and certain non-adipocytic soft tissue sarcomas. SARC024 is a blanket protocol to study oral regorafenib in patients (pts) with selected sarcoma subtypes. The primary objective of cohort D was to estimate the RECIST 1.1 progression free survival (PFS) rate in pts with rhabdomyosarcoma (RMS) at 8 and 16 weeks (wks). The secondary objectives were to estimate the RECIST 1.1 objective response rate (ORR) at 8 and 16 wks as well as describe the incidence of adverse events secondary to regorafenib.

Methods: Pts ≥5 years (yrs) of age and with the ability to swallow pills, diagnosed with relapsed or refractory embryonal RMS (ERMS) or alveolar RMS (ARMS) with measurable disease according to RECIST 1.1 and had received at least one prior line of systemic chemotherapy, and had recovered from acute toxicities of previous anti-cancer therapies, and with adequate organ function and performance status were included after giving written informed consent. Pts with active CNS disease, severe or uncontrolled medical disease including hypertension, active heart disease, bleeding, thrombosis, seizures, interstitial lung disease, and malabsorption were excluded, as were pregnant or breast-feeding women and those with history of prior treatment with small molecule oral tyrosine kinase inhibitors. Regorafenib was administered once daily orally in 4-week cycles at a dose of 160 mg (82 mg/m2/dose for pts < 18 years of age rounded to the nearest 20 mg) for 3 wks on and 1 wk off and could continue indefinitely until disease progression or physician/pt decision to stop treatment. ORR was measured at 8 wks and 16 wks according to RECIST 1.1 and PFS as time to progression (TTP) from enrollment. Disease control of 30% at 8 wks and ORR of 30% at 8 weeks in a sample size of up to 12 pts was required to consider regorafenib efficacious. Toxicity was assessed according to NCI CTCAE v.4.03.

Results: Ten eligible pts were enrolled between 21Mar2018 and 7Sep2021 at 5 different institutions; 5 were male and 5 were female; median age was 26 yrs (range 16, 63). Eight had metastatic disease There was one grade (gr) 5 pleural effusion secondary to disease progression. There were no unexpected toxicities with single episodes of gr 3 increased ALT, anemia, hypophosphatemia, maculopapular rash, vomiting and single episodes of gr 4 decreased neutrophils, decreased WBC and decreased platelets observed. Nine of 10 patients were evaluable for response and 3 had a best response of stable disease while the remaining 6 had progressive disease. The PFS at 8 wks was 50% (95% CI 26.9-92.9%) and declined to 20% (95%l CI 5.8-69%) at 16 wks.

Conclusion: Regorafenib as a single agent has limited activity in relapsed/refractory RMS. There were no objective responses observed in pts with either ERMS or ARMS enrolled in cohort D of SARC024. PFS exceeded the bar for efficacy set by the trial at 8 weeks but was not sustained at 16 weeks. The EpSSG FAR-RMS trial is investigating the combination of oral regorafenib together with intravenous vincristine and irinotecan in pts with relapsed/refractory RMS (NCT04625907).

(P 192) ROLE OF MAGNETIC RESONANCE IMAGING IN THE PREDICTION OF HISTOLOGICAL GRADE IN SOFT TISSUE SARCOMAS.

Tomás M D M Marques, MD / MSc[1]; Wagner Cerqueira, MD[2]; Celso A L Mello, Ph.D[2]; João Flores Neto, MD[2]; Tiago Bezerra, MD[2]; Paulo Stevanato, Ph.D[2]; Renata Takahashi, MD[2]; Bruna Catin, n/a[2]; Suely Nakagawa, MD[2]; Wilson Nakagawa, Ph.D[2]; Maria Leticia Silva, MD[2]; Fernando Campos, MD[3]; Felipe Costa, Ph.D[2]; Antonio Nascimento, Ph.D[2]; Ademar Lopes, Ph.D[2]; Samuel Aguiar Jr., Ph.D[2], (1) AC Camargo Cancer Center - Sao Paulo - Brazil, São Paulo, Sao Paulo, Brazil, (2) AC Camargo Cancer Center, São Paulo, Sao Paulo, Brazil, (3) Medical Oncology Department, A.C.Camargo Cancer Center, São Paulo, SP, Brazil, Sao Paulo, Sao Paulo, Brazil

Objective: This study aims to assess the role of pre-operative Magnetic Resonance Imaging (MRI) in predicting the histological grade of non-metastatic soft tissue sarcoma.

Methods: This is an observational, cross-sectional retrospective single center study. The inclusion criteria consisted of patients over 18 years old who underwent guided core needle biopsy or external pathologic diagnosis review, MRI examination, and primary tumor surgery at the ACCCC between January 2015 and June 2022. Patients who underwent surgical biopsy were excluded. The main variables included age, tumor size, location, histological subtype, histological grade of the biopsy and surgical specimen (categorized as high grade for G2 and G3 and low grade for G1 of the FNCLCC classification). Two radiologists from our institution evaluated MRI radiological criteria such as size, margin definition (regular or irregular), heterogeneity of the T2 signal (greater than 50% or less than 50%), high intensity peritumoral signal on T2 (presence or absence of peritumoral edema), and peritumoral post-contrast enhancement (presence or absence). Additionally, a score was calculated to assess the prediction of histological grade by summing the positive criteria analyzed (ranging from 0 (zero) when no criteria were identified to 5 (five) when all criteria were positive). The Chi-square and Fisher's exact test were employed to examine the relationship between the MRI parameters and the histological grade of the surgical specimen and logistic regression analysis to identify independent associations was performed. A p-value < 0.05 was considered statistically significant.

Results: A total of 68 patients who met the inclusion and exclusion criteria were included in the study, comprising 30 women and 38 men. The biopsy identified 54 high-grade tumors and 14 low-grade tumors. In the surgical specimen, 52 high-grade tumors and 16 low-grade tumors were observed. The most common type of biopsy was ultrasound-guided core needle biopsy, accounting for 54.4% of cases. The most frequent histologies were Liposarcoma (26.4%), followed by synovial sarcoma (17.6%) and leiomyosarcoma (10.3%). The majority of tumors were located in the extremities (64.7%), and tumors larger than 5cm were identified in 89.7% of cases. Margins were regular in 43 (63.2%) cases and irregular in 25 (36.8%) cases. Tumor heterogeneity in T2 was observed in 36 (52.9%) cases compared to 32 (47.1%) cases without heterogeneity. Peritumoral high-intensity T2-weighted signal was present in 45 (66.2%) cases, while peritumoral post-contrast enhancement was present in 31 (45.6%) cases. The scores were as follows: 2 with a score of 0 (zero), 10 with a score of 1 (one), 16 with a score of 2 (two), 13 with a score of 3 (three), 18 with a score of 4 (four), and nine with a score of 5 (five). The MRI criteria that demonstrated an association with histological grade were peritumoral high-intensity T2-weighted signal (p < 0.001) and peritumoral post-contrast enhancement (p = 0.006). Logistic regression analysis indicated that the presence of peritumoral high-intensity T2-weighted signal (OR 11.8, CI 95% 3.0 – 41.5, p < 0.001) and peritumoral post-contrast

enhancement (OR 8.8, CI 95% 1.8 - 42.8, p 0.007) were also predictive of high-risk tumors. Although the other MRI criteria were not associated with histological grade, applying the score (sum of the MRI findings) revealed that each additional point doubled the chance of a high-grade tumor (OR 2.0; CI 95% 1.2 – 3.3, p = 0.005).

Conclusion: MRI is an effective tool for predicting the histological grade of soft tissue sarcomas. MRI criteria related to the infiltrative tumor growth pattern, such as peritumoral high-intensity T2-weighted signal and peritumoral post-contrast enhancement, showed positive predictive value for histological high grade. These findings could be helpful in better defining the treatment plan. Further studies should validate our data.

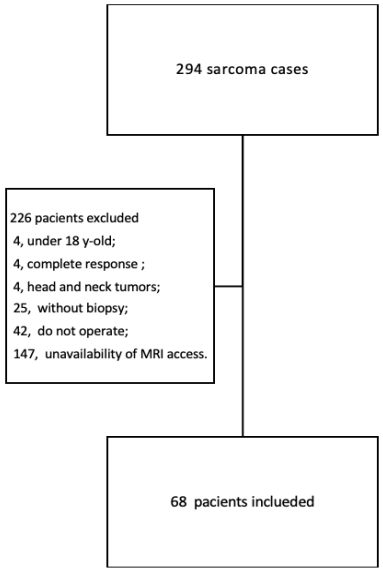


Figure 1: Flowchart of inclusion of patients in the study

Table 1: Descriptive distribution of patients included in the study.

Characteristic	Frequency	%
Gender		
Male	39	59.9
Female	30	44.1
Age		
<45 yo	27	39.7
>45 yo	41	60.2
Location		
Extremity	44	64.7
Lower limb	31	70.4
Upper limb	13	29.5
Trunk	16	23.5
Retroperitoneum	8	11.8
Depth		
Superficial	10	14.7
Deep	50	73.5
Tumor Size		
< 5cm	7	10.3
> 5cm	61	89.7
Stage		
T1	7	10.3
T2	31	45.6
T3	12	17.6
T4	13	19.1
N0	67	98.5
N1	1	1.5
M0	61	89.7
M1	7	10.3
Histologic type		
Dedifferentiated liposarcoma	7	10.3
Myxoid Liposarcoma	3	4.4
Pleomorphic Liposarcoma	2	2.9
Liposarcoma	6	8.8
Fibrosarcoma	2	2.9
Myxofibrosarcoma	1	1.5
Pleomorphic sarcoma	4	5.9
Leiomyosarcoma	7	10.3
Rhabdomyosarcoma	4	5.9
Malignant peripheral nerve sheath tumor	1	1.5
Synovial Sarcoma	13	19.1
Sarcoma Alveolar	1	1.5
Spindle cell sarcoma	4	5.9
Carcinosarcoma	1	1.5
Other	13	19.1
ASA		
ASA 1	10	14.7
ASA 2	47	69.1
ASA 3	10	14.7
ASA 4	1	1.5
ASA 5	0	0
ECOG		
ECOG 0	23	33.8
ECOG 1	43	63.2
ECOG 2	3	4.4
ECOG 3	0	0
ECOG 4	0	0
Comorbidities		
Yes	37	54.4
No	31	45.6
Histological grade Biopsy		
High grade	54	79.4
Low grade	14	20.6
Final Histological grade (surgery)		
High grade	52	76.5
Low grade	16	23.5
Clinical stage		
Localizated	56	82.4
Metastatic	12	17.6
Treatment approach		
Surgery	24	35.3
Neoadjuvant therapy	41	60.3
Neoadjuvant chemotherapy	9	21.9
Neoadjuvant radiotherapy	17	41.4
Neoadjuvant chemo and radiotherapy	10	24.3
Neoadjuvant chemoradiation	5	12.1
Previous Treatment		
ACCC	3	4.4
External	6	8.8
Biopsy		
Tomography-guided	7	10.3
Ultrasound-guided	37	54.4
Made review	23	33.8
Colonoscopy	1	1.5
Immunohistochemistry		
Biopsy	53	77.9
Surgery	9	13.2

Table 2: Descriptive distribution of patients in relation to MRI features.

Characteristic	Frequency (n)	%
Tumor Size		
< 5 cm	7	10.3
> 5cm	61	89.7
Margin definition		
Regular	43	63.2
Irregular	25	36.8
Heterogeneity T2 sign on T2-weighted sequences.		
> 50%	36	52.9
< 50%	32	47.1
High-intensity peritumoral sign on T2-weighted sequences.		
Yes	45	66.2
No	23	33.8
Peritumoral post-contrast enhancement		
Yes	31	45.6
No	37	54.4
Score RNM		
0	2	2.9
1	10	14.7
2	16	23.5
3	13	19.1
4	18	26.5
5	9	13.2

Table 3: Associations between Conventional MRI Features and Histologic Final Grade.

Characteristic	Low Grade n (%)	High Grade	P value	Odd ratio (CI95%)*
Tumor size			1.000	
< 5cm	1 (1.5%)	6 (8.8%)		
> 5cm	15 (22%)	46 (67.6%)		
Margin Definition			0.412	
Regular	12 (17.6%)	31 (45.5%)		
Irregular	4 (5.9%)	21 (30.8%)		
Heterogeneity of the T2 sign on T2-weighted			0.578	
> 50%	7 (10.3%)	29 (42.6%)		
< 50%	9 (13.2%)	23 (33.8%)		
High-intensity peritumoral sign on T2-weighted sequences.			0.000	11.1 (3.0 – 41.5)
Yes	4 (5.9%)	41 (60.2%)		
No	11 (16.2%)	12 (17.6%)		
Peritumoral post-contrast enhancement			0.006	8.8 (1.8 – 42.8)
Yes	2 (2.9%)	29 (42.6%)		
No	14 (20.5%)	23 (33.8%)		
Score RNM			0.014	2.0 (1.2 – 3.3)
0	0	2 (2.9%)		
1	6 (8.8%)	4 (5.8%)		
2	6 (8.8%)	10 (14.7%)		
3	2 (2.9%)	11 (16.2)		
4	2 (2.9)	16 (23.5%)		
5	0	9 (13.2%)		

* Odds ratio (95% confidence interval) after logistic regression analysis

(P 193) **Recent Trends in Sarcoma Trials for Children in the United States**

Jonathan L. Metts, MD[1]; Ajay Gupta, MD, MS[2]; Maua Mosha, N/A, MPH[3]; Alexandra Miller, BS[3]; Jennifer Jones, RN[3]; Lynda Beaupin, MD[3], (1) Moffitt Cancer Center and Johns Hopkins All Children's Hospital, Tampa, FL, (2) Roswell Park Oishei Children's Cancer and Blood Disorders Program, Buffalo, NY, (3) Johns Hopkins All Children's Hospital, St Petersburg, FL

Objective: Sarcomas require new therapies to improve cure rates. Sarcomas occur in children and adults, thus inclusion of children (patients under 18 years of age) in sarcoma studies is critical. Our objective was to evaluate recent sarcoma trials enrolling children to determine temporal trends in therapeutic trials available to children with sarcoma in the United States (US).

Methods: Using sarcoma-focused search terms, ClinicalTrials.gov was queried for studies 1) with a lower-bound age limit ≤17 years, regardless of upper age limit, 2) identified as interventional, 3) with a study start between September 27th, 2007 and January 11th, 2023, and 4) with ≥1 site in the US. Studies were categorized by cancer type, intervention type, age range eligible, funding source, study location, number of sites, and study status. Trials were examined as a whole and stratified to compare 1) Pediatric trials (defined here as enrolling ≤21 years of age) vs. Adult/Pediatric trials (enrolling >21 years of age) and 2) trials receiving industry funding vs. no industry funding. Continuous variables were compared by parametric or non-parametric tests according to the sample distribution. Categorical variables were compared using Chi-square tests or Fisher's exact test. All statistical tests were two-sided and a p-value < 0.05 was considered statistically significant. The Mandal-Kenn test was used to investigate temporal trends in trial frequency.

Results: Of 273 trials included, 215 (79%) were pediatric/adult while 58 (21%) were pediatric.

The most common cancer types were mixed (n=70, 25%), multiple solid tumors (n=64, 23%), and multiple sarcoma types (n=59, 22%). Most trials were Phase 1/2 or Phase 2 (n=161, 59%). The most common intervention was drug/biologic agents (n=199, 73), while 31 trials (11%) involved stem cell transplant/cell therapy and 31 trials (11%) used multiple interventions. Most trials received no industry funding (n=155, 57%), were exclusive to the US (75%), and were multi-institutional (n=180, 66%). Eighty-three trials (30%) were completed with a median duration of 4 years (range 0.8-9.8 years). Twenty-three trials (8.4%) were terminated, most commonly due to slow accrual (39%) and drug development decision (26%). Only five trials enrolled exclusively patients < 18 years old; all were Phase 1 or 2, all studied drug/biologic therapies, and three of five were industry-supported. Pediatric trials were more likely to enroll mixed cancer types, while pediatric/adult studies were more likely to enroll multiple sarcoma types or focus on specific sarcomas. Similar proportions between these groups were seen across trial phase, funding source, intervention, number of sites, study location, and reason for termination. A non-statistically significant trend was noted towards shorter trial duration for pediatric studies (3.65 vs 4.8 years, P=.08). Studies with industry funding were more likely to be Phase 1/2 or Phase 2 (69% vs. 51%, P=.002). Industry-funded studies had proportionately more drug/biologic interventions 86% vs. 63%, p<.001), were more likely to be international (41% vs. 14%, P=.002) and more likely to be multi-site (76% vs. 58%, P=.002). New sarcoma trial activations increased by year from 2008 through 2022 (P=0.01). A median of 18 (range 10 to 27) sarcoma trials activated each year, peaking in 2017. No specific yearly trends were noted for trial phase, intervention type, or funding source.

Conclusion: Clinical trials enrolling children with sarcoma most often span pediatric and adult ages, are multi-institutional, and have low termination rates, most often due to slow accrual. The number of new trials increased yearly. Most trials did not receive industry funding, though those funded by industry were more likely to be early phase efforts studying novel drug or biologics and enrolling at multiple sites and internationally.

(P 194) REAL WORLD OUTCOMES OF PATIENTS TREATED WITH GEMCITABINE AND DOCETAXEL FOR ADVANCED SOFT TISSUE SARCOMA IN AN AUSTRALIAN SARCOMA CENTRE

Isabella Wilson, MD[1]; Madeleine Strach, MBBS, FRACP[2]; Vivek A. Bhadri, MD, PhD[2]; Michelle Harrison, MBBS FRACP[2]; Whiter Tang, Pharmacist[2]; Peter Grimison, MD, PhD[3], (1) Chris O'Brien Lifehouse, Neutral Bay, New South Wales, Australia, (2) Chris O'Brien Lifehouse, Sydney, New South Wales, Australia, (3) Chris O'Brien Lifehouse, SYDNEY, New South Wales, Australia

Objective: Gemcitabine and docetaxel (GD) is a standard chemotherapy regimen for soft tissue sarcoma (STS). The GeDDiS phase 3 trial compared GD with doxorubicin in the 1st-line setting, using a 90-minute infusion time for gemcitabine – reporting a response rate of 20%, progression-free survival (PFS) (median, 6-month rate) of 5.4 months and 46%, and most common grade 3/4 toxicity of neutropenia (25%) and febrile neutropenia (12%) (1). We aimed to examine the real-world efficacy and toxicity of GD in our centre in the 1st and later line using a 30-minute infusion time for gemcitabine.

Methods: A retrospective analysis was conducted of patients with unresectable or metastatic STS receiving ≥ one cycle of GD between July 2018 – October 2022. Data collected included

patient and tumour characteristics, dose intensity, toxicity, response, PFS and overall survival (OS). Survival outcomes were calculated from the date of 1st GD treatment using the Kaplan Meier Method.

Results: 38 patients were included for analysis (Table 1). Median follow-up was 19 months (range 3-30). Histologic subtypes (n) were leiomyosarcoma (20), synovial sarcoma (4), undifferentiated pleomorphic sarcoma (4), other (10). Primary sites (n) were extremity (12), uterine (8), other pelvic (7), intra-thoracic (4), retroperitoneal (3), other (4). Line of treatment (n) was 1st line (10), 2nd line (13), ≥ 3rd line (15). Median number of cycles was 6 (range 1-12). 16 patients (42%) required dose modification during the 1st 3 cycles. Response rate was 42%, including 5% with complete response (Table 2). At the time of data collection, 33 patients had disease progression and 24 patients had died. PFS (median, 6-month rate) was 4.5 months and 44% (Figure 1). Overall survival (median, 12-month rate) was 15 months and 65% (Figure 2). Grade 3/4 toxicity included neutropenia (8%), febrile neutropenia (3%) and anaemia (21%). There was 1 death possibly attributable to GD.

Conclusion: This data demonstrates efficacy of GD comparable to other published data and favourable toxicity in a real-life patient population despite shorter gemcitabine infusion time and heavily pre-treated patient population.

Table 1: Baseline Characteristics

Characteristic	N (%)
Gender	
F	25 (66%)
M	13 (34%)
Age at time of commencement of GD (median)	54 (Range 18-80)
ECOG	
0	9 (24%)
1	26 (68%)
2	3 (8%)
Histology	
Leiomyosarcoma	20 (53%)
Undifferentiated pleomorphic sarcoma	4 (11%)
Synovial sarcoma	4 (11%)
Liposarcoma	1 (2%)
Other	9 (23%)
Primary Site	
Extremity	12 (32%)
Retroperitoneal	3 (8%)
Uterine	8 (21%)
Pelvic (non-uterine)	7 (18%)
Intra-thoracic	4 (10%)
Other	3 (8%)
Unknown	1 (3%)
Disease status at initial diagnosis	
Resectable	22 (58%)
Unresectable	2 (5%)
Metastatic	14 (37%)
Prior radiotherapy	
Yes	17 (45%)
No	21 (55%)
Prior lines of systemic therapy	
0	10 (26%)
1	13 (34%)
2	11 (29%)
3+	4 (11%)

Table 2 Efficacy Outcomes

Endpoint	N (%)	Median (95% CI), months	6-month	12-month
Best response		-	-	-
Progression	10 (26%)			
Stable	12 (32%)			
Partial	14 (37%)			
Complete	2 (5%)			
Progression-free survival	38 (100)	5 (4-7)	44%	19%
Overall survival	38 (100)	15 (14-21)	79%	65%

Figure 1: Progression Free Survival

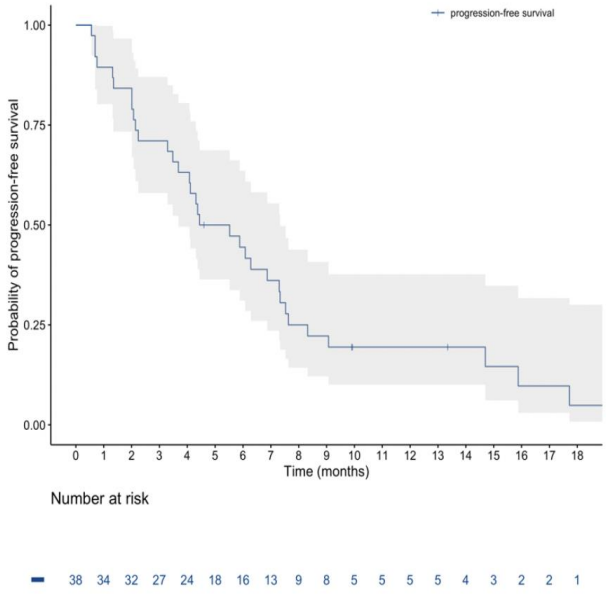
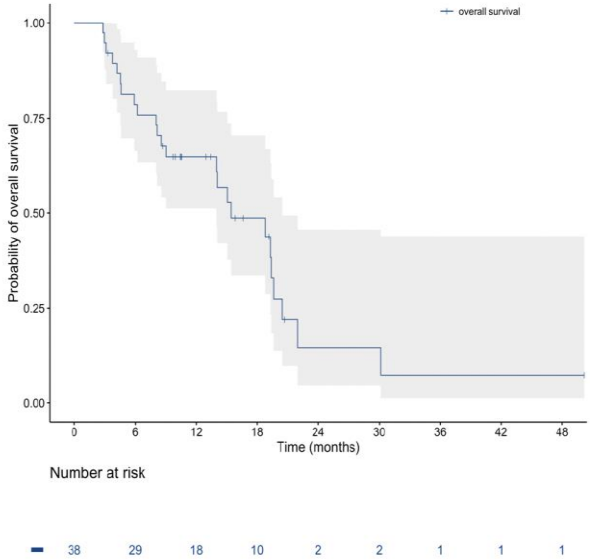


Figure 2: Overall Survival



(P 195) MULTIPLE MYXOMA EFFECTIVELY TREATED WITH PAZOPANIB - A CASE REPORT

Mikael Eriksson, MD, PhD[1]; Marie Ahlstrom, MD[2], (1) Skane University Hospital, Lund, Skane Lan, Sweden, (2) Dpt of Oncology, Skane University Hospital, Lund, Sweden, Lund, Skane Lan, Sweden

Objective: Benign mesenchymal tumors rarely need medical treatment, but there may be cases where such tumors give rise to severe symptoms but are not possible to remove by surgery because of advanced growth adjacent to vital organs or multiplicity. Such a case is described here focusing on the different methods tried to alleviate severe and disabling symptoms in a seemingly unique condition. Several methods and drugs failed, but finally a treatment attempt was made with the tyrosine kinase inhibitor pazopanib, which has a demonstrated effect in several cases of soft tissue sarcomas. The slow but steady effect of this treatment may be of interest for other rare cases of benign but not operable mesenchymal tumors.

Methods: A 44-year old woman noticed a nodule on her left thigh in March 2009. Some months later similar nodules were noticed in the groins on each side. An MRI showed multiple intramuscular tumors, also at other locations. Excisional biopsy was performed from two of the lesions showing intramuscular myxoma without any signs of malignancy. With time, several tumors were growing giving rise to pain, gait difficulties, and sick leave. In September 2010, surgical removal was performed of two of the largest lesions, but fast re-growth did occur postoperatively. In May 2011, radiotherapy was used against one lesion in the right thigh with a transient effect of about one month. It was decided to start experimental medical therapy, and with no earlier reports in the literature of a similar condition, we tried in order, anti-estrogenic

therapy with toremifen, mTOR-inhibition with everolimus, and interferon, before starting TKI treatment with pazopanib. At start of the last therapy, she had 14 measurable lesions of up to 30 cm in length.

Results: No, or only very limited, effects were registered after toremifen, everolimus, and interferon. After start of pazopanib no further progress was seen, and after three months some of the lesions became softer. After 8 months a marked regression of all lesions was found, the largest now measuring 22 cm, and only 5 were still measurable. One year after start the largest was only 12 cm, and 3 months later there was no measurable lesion left. MRI verified an almost complete regression. Some side effects encouraged us to a slow stepwise dose decrease during several months, but when we finally stopped treatment completely, a regrowth was obvious in several sites. Retreatment, however, led to a repeated extremely good effect, and the patient has now been on low doses of pazopanib during more than ten years, between 200 and 400 mg daily, which has been well tolerated and without occurrence of any worrying long-time side effects.

Conclusion: This rare case of multiple intramuscular myxomas demonstrates that VEGF-directed TKI, e.g., pazopanib, may be an excellent treatment modality also in benign mesenchymal tumors.

(P 196) TOLERABILITY AND OUTCOMES FOR TREATMENT OF OLDER MYXOID LIPOSARCOMA POPULATION

Thanh P. Ho, MD[1]; Reilly A. Coombs, M.S.[2]; Judith Jebastin Thangaiah, M.B.B.S., MD[1]; Brittany Siontis, MD[1]; Steven Robinson, MBBS[1]; Scott Okuno, M.D.[1]; Matthew T. Houdek, MD[1], (1) Mayo Clinic, Rochester, MN, (2) Medical College of Wisconsin-Central Wisconsin, Wausau, WI

Objective: Myxoid liposarcoma (MLPS) has highest incidence in middle age. Little is known regarding treatment and outcomes in older patients (pts). This study aims to better understand this older pt population.

Methods: Chart review was performed of pts aged 70 years and above with non-metastatic MLPS between 1992 to 2021 who were seen at an academic sarcoma center. Analysis was performed on pts with at least two years (yrs) of follow up.

Results: Twenty pts with MLPS were identified, with a median age of 74 yrs (range 70 to 89) at diagnosis (Table 1). Over half were female (11, 55%). Most tumors were extremity in location (16 pts, 80%) with median tumor size of 11 cm (3.6 to 30 cm). Seven pts (35%) had round cell component (percentage not reported).

Eighteen of 20 pts (90%) received perioperative radiation. Over half had postoperative radiation (10 pts, 55%). One of 8 pts who received preoperative radiation developed metastasis prior to planned resection. Only 1 of 19 patients who underwent surgery developed a postoperative wound complication. Three pts (15%) with high grade disease received perioperative chemotherapy.

The median follow-up from surgery was 75 months (mos) (5-239). Eleven (55%) pts died from MLPS. The median progression free and overall survival were 39 mos (3-76) and 62 mos (5-

108), respectively. Four of 7 pts (57%) with a round cell component died from their disease.

The median survival after relapse was 9 mos (0-32). Ten pts had distant metastasis (most commonly to bone), and one had local relapse (to retroperitoneum). Two pts (18%) had additional surgery. Five (45%) had RT and six (55%) had systemic therapy for relapse. Pts had minimal side effects from RT, mainly fatigue, gastrointestinal side effects, and erythema of RT site. Systemic therapies included: doxorubicin alone or in combination with ifosfamide, olaratumab, or mitomycin C/cisplatin; trabectedin, gemcitabine, ifosfamide/etoposide, nivolumab. One patient stopped trabectedin due to toxicity and was hospitalized due to gemcitabine toxicity; otherwise, systemic therapies were well tolerated, with fatigue, anorexia, and emesis commonly reported.

Conclusion: MLPS is considered a chemotherapy and RT sensitive sarcoma subtype. In this cohort of pts age ≥70 yrs with MLPS, the majority received surgery with perioperative radiation therapy. Few pts received perioperative chemotherapy for localized high grade MLPS, precluding meaningful analysis. Pts who received systemic therapy for recurrent disease tolerated treatment well, and systemic therapy should be considered for older pts with advanced disease.

Table 1

	Extremity (n=16)	Trunk (n=2)	Retroperitoneum (n=2)	Total (n=20)
Female, n (%)	10 (63%)	1 (50%)	0 (0%)	11 (55%)
Median age, years (range)	75.5 (70-89)	70.5 (70-71)	72	74 (70-89)
Median tumor size, cm	10.4 (3.6-28.0)	10.8 (10.0-11.5)	24.8 (19.5-30.0)	11.0 (3.6-30.0)
Radiation therapy*	Preoperative: 6 Postoperative: 8	Preoperative: 1 Postoperative: 1	Preoperative: 1 Postoperative: 1	Preoperative: 8** Postoperative: 10
Perioperative chemotherapy, n (%)	1 (0.1%)†	2 (100%)††	0 (0%)	3 (15%)
Recurrence, n (%)	9 (56%)	1 (50%)	1 (50%)	11 (55%)
Time from surgery to recurrence, months (range)	47 (3-76)	15 months	39 months	39 (3-76)
Surgery for recurrence, n (%)	0 (0%)	1 (50%)	1 (50%)	2 (10%)
Radiation therapy for recurrence, n (%)	5 (56%)	0 (0%)	0 (0%)	5 (25%)
Systemic therapy for recurrence, n (%)	4 (44%)	1 (50%)	0 (0%)	6 (30%)

*Eleven pts had radiation dose reported: median 50.4 Gray (range 32-64.8).
**Two pts received preoperative and intraoperative radiation therapy.
†One pt received preoperative ifosfamide/etoposide and was hospitalized for neutropenic fever.
††One pt received preoperative cisplatin with radiation therapy. One pt received postoperative doxorubicin/ifosfamide. Neither had complications.

(P 197) PROPRANOLOL MONOTHERAPY IN ANGIOSARCOMA - A WINDOW-OF-OPPORTUNITY STUDY (PROPANGIO)

Alaa Embaby, MD[1]; Kimberley M. Heinhuis, MD, PhD[1]; Nikki Ijzerman, Dr.[2]; Anne Miek Koenen, n/a[2]; Stephanie van der Kleij, n/a[1]; Ingrid Hofland, n/a[1]; Hester H. van Boven, Dr.[1]; Joyce Sanders, MD[1]; Winette van der Graaf, Prof. Dr.[2]; Rick Haas, MD, PhD[3]; Alwin D.R.

Huitema, Prof. Dr.[1]; Neeltje Steeghs, Dr.[2]; Winan J. van Houdt, MD, PhD, MS[2], (1) Netherlands Cancer Institute/Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (2) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (3) The Netherlands Cancer Institute, Amsterdam, Drenthe, Netherlands

Objective: Angiosarcoma is a rare and aggressive cancer of the endothelial cells. It has been shown that β-adrenergic signaling through the β-adrenoceptors (ADRB) 1 and 2, that are overexpressed in angiosarcoma, promotes tumor growth. Propranolol, a non-selective β-blocker, was able to initiate apoptosis in angiosarcoma cell lines and its anti-tumor activity has been demonstrated in several case reports. The aim of this window-of opportunity trial was to prospectively evaluate the anti-tumor activity of propranolol in patients with angiosarcoma.

Methods: Patients with angiosarcoma with a waiting time of 3 to 6 weeks until start of standard treatment were eligible. Propranolol was dosed 80 mg to 240 mg/day according to a dose titration schedule. The primary endpoint was clinical response rate. Clinical response was defined as either complete response (CR), partial response (PR) (according to RECIST 1.1) or stable disease (SD) with improvement of erythema, edema and thickness. Exploratory objectives included histologic response, PET response and assessment of potential biomarkers for response in tumor tissue by IHC (e.g. ADBR 1/2, immune system-based biomarkers).

Results: To date, 13 patients with a median age of 67 years (range 52-86) are enrolled and have initiated treatment. The median duration of treatment was 28 days (range 21-42 days). The median dose was 160 mg/day. Three patients showed exceptional good tolerance (blood pressure/heart rate) and could be escalated to the highest dose (240 mg/day). Two patients showed clinical response. The most common adverse event was grade 1/2 bradycardia (85%). There were no grade ≥3 adverse events. Preliminary IHC results showed ADBR2 overexpression in the majority of the tumors (12 out of 17 tumor samples, both in responders and non-responders). None of the tumors showed ADBR1 overexpression.

Conclusion: This window-of-opportunity trial did not demonstrate clinical efficacy of propranolol monotherapy in patients with angiosarcoma. Two out of 13 patients did however show clinical benefit. ADBR1 or 2 expression did not correlate with response. Translational research to better comprehend the anti-tumor activity of propranolol for adequate selection of patients in future studies is ongoing.

(P 198) PERSONALIZED MULTIDISCIPLINARY MANAGEMENT OF SARCOMA IN PATIENTS WITH LYNCH SYNDROME

Ryan A. Denu, MD, PhD[1]; Sintawat Wangsiricharoen, MD[2]; Davis R. Ingram, BS[2]; Khalida Wani, PhD[2]; Alexander J. Lazar, MD, PhD[2]; Christina Roland, MD, MS[2]; Yi-Qian N. You, MD, MHSc[1], (1) MD Anderson Cancer Center, Houston, TX, (2) The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: Lynch syndrome is an autosomal dominant cancer predisposition syndrome caused by a germline loss-of-function mutation in one of the DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, and PSM2). Sarcomas are not classically thought of as Lynch-associated cancers but do occur in patients with Lynch syndrome. This project aimed to assess

whether these sarcomas are related to Lynch syndrome and display loss of MMR proteins and/or microsatellite instability (MSI) and to determine the optimal treatment of this unique patient population.

Methods: We included 30 patients with a diagnosis of Lynch syndrome (confirmed by germline genetic testing) with a history of sarcoma seen at MD Anderson Cancer Center with sarcoma diagnosis between 1998 and 2022. To assess for MMR deficiency, immunohistochemistry was performed on the sarcoma specimens to assess for expression of MMR proteins. If the staining was normal or intact, we assessed for MSI by PCR assay, as some loss-of-function MMR gene mutants will retain a normal expression pattern. We also extracted and analyzed available clinical data from their medical records.

Results: Among 30 patients with Lynch syndrome and sarcoma, germline mutation in MSH2 was most common (50%), followed by MSH6 (20%), PMS2 (16.7%), and MLH1 (13.3%). The most common sarcoma subtypes in this cohort were undifferentiated pleomorphic sarcoma (40%) and leiomyosarcoma (26.7%). Median age at diagnosis was 49.2 years (range 11.2-79.3). Tissue was available for MMR/MSI testing in 10 cases. Deficient MMR or MSI was demonstrated in 4 of these 10 (40%); One case had intact MMR and stable microsatellite (case 8); MSI testing will be performed on the remaining 5 with intact MMR protein expression. Three patients were treated with immunotherapy. One patient treated with ipilimumab and nivolumab, had a partial response, and was on therapy for 31 months until death from an unrelated cause. The second patient has been on pembrolizumab monotherapy for 44 months and has had a complete response. The third patient treated with pembrolizumab monotherapy developed progressive disease after 4 months. With a median follow-up time of 68.2 months since sarcoma diagnosis (range 2.5-262.7 months), median overall survival and progression-free survival have not been reached.

Conclusion: While rare, Lynch syndrome can be associated with sarcoma. This is most commonly seen with germline MSH2 mutation and undifferentiated pleomorphic sarcoma and leiomyosarcoma subtypes. Immunotherapy has the potential for robust and durable responses and the potential to minimize or obviate surgery in Lynch-associated sarcomas. This is important to consider in this unique patient population that has had or potentially will require treatment for additional malignancies.

Characteristics of 30 patients with Lynch syndrome and sarcoma

ID	Lynch gene	Mutation	Pathogenic?	Sarcoma type	Other Cancers	MMR/MSI analysis of sarcoma	Immunotherapy?	Response to Immunotherapy
1	MLH1	c.790+4A>C (splice site)	Variant Suspected Deleterious	Spindle cell sarcoma, NOS	Colorectal	-		
2	MLH1	Exon 6-12 duplication	Variant, Likely Deleterious	Unknown	Esophageal, pancreatic	-		
3	MLH1	c.1381A>T (p.K461*)	Positive	UPS	Colon, sebaceous adenocarcinoma	-		
4	MLH1	c.1852_1854delAAG (p.K618del)	Positive	UPS	Rectal	Intact MLH1		
5	MSH2	c.1361T>G (p.I454R)	Variant Suspected Deleterious	UPS	Bladder, colon, gastric	-		
6	MSH2	c.158C>G (p.A59G)	Variant of Uncertain Significance	High grade sarcoma	None	-		
7	MSH2	c.170T>G (p.V57G)	Variant of Uncertain Significance	Ewing sarcoma	Papillary thyroid, adrenal cortical adenoma	-		
8	MSH2	c.368C>G (p.A123G)	Variant of Uncertain Significance	Leiomyosarcoma	Melanoma, prostate	Intact MSH2		
9	MSH2	Exons 1-6 deletion	Positive	Rhabdomyosarcoma	Endometrial	Loss of MSH2 and MSH6; MSI by PCR	Yes	PR with ipilimumab/nivolumab for 31 months.
10	MSH2	c.1387-4G>C (splice site)	Variant of Uncertain Significance	Leiomyosarcoma	Breast	-		
11	MSH2	c.1906G>C (p.A636P)	Positive	Endometrial stromal sarcoma	None	-		
12	MSH2	Exons 9-16 deletion	Positive	Pleomorphic rhabdomyosarcoma	Colon, bladder	MSI by PCR	Yes	CR with pembrolizumab in metastatic setting.
13	MSH2	c.716A>T (p.Q239L)	Variant of Uncertain Significance	UPS	Rectal, breast	Intact MSH2		
14	MSH2	c.942+3A>T (splice site)	Positive	UPS	Colon, gastric, glioblastoma	-		
15	MSH2	Exon 1-6 deletion	Positive	Pleomorphic liposarcoma	Colon, SCC, MDS	Loss of MSH2		
16	MSH2	c.2320A>G (p.I774V)	Variant of Uncertain Significance	Osteosarcoma	Rectal	Intact MSH2		
17	MSH2	c.942+3A>T (splice site)	Positive	Pleomorphic spindle cell sarcoma	Colorectal	Loss of MSH2		
18	MSH2	c.2291G>A (p.W764*)	Positive	Leiomyosarcoma	None	-		
19	MSH2	Complex deletion involving EPCAM and MSH2 exons 1-7	Positive	Malignant epithelioid and spindle cell neoplasm	Colon	-		
20	MSH6	c.1915G>A (p.E639K)	Variant of Uncertain Significance	Leiomyosarcoma	Neuroblastoma, RCC, pheochromocytoma, melanoma, BCC	-		
21	MSH6	c.3699_3702delAGAA (p.K1233fs)	Positive	UPS	Colon, prostate	-		
22	MSH6	c.1354A>G (p.M452V)	Variant of Uncertain Significance	Epithelioid sarcoma	None	-		
23	MSH6	c.2983G>A (p.E995K)	Variant of Uncertain Significance	Spindle cell sarcoma	Gallbladder	-		
24	MSH6	c.3991C>T (p.R1331*)	Positive	High grade pleomorphic sarcoma	Colorectal	-		
25	MSH6	c.2057G>A (p.G686D)	Positive	Leiomyosarcoma	Seminoma	-		
26	PMS2	Whole gene deletion	Positive	Leiomyosarcoma	None	-	Yes	PD with pembrolizumab after 4 months
27	PMS2	c.1A>G (p.M1V)	Positive	Ewing sarcoma	DLBCL	Intact MMR; MSS by PCR		
28	PMS2	c.1169C>T (p.A390V)	Variant of Uncertain Significance	Synovial sarcoma	None	-		
29	PMS2	c.2012C>T (p.T671M)	Variant of Uncertain Significance	Leiomyosarcoma	Breast	-		
30	PMS2	c.24-1G>A (splice site)	Positive	Leiomyosarcoma	None	Intact PMS2		

-, not performed; BCC, basal cell carcinoma; DLBCL, diffuse large B-cell lymphoma; MDS, myelodysplastic syndrome; NOS, not otherwise specified; RCC, renal cell carcinoma; SCC, squamous cell carcinoma; UPS, undifferentiated pleomorphic sarcoma

(P 199) **PEXIDARTINIB RISK EVALUATION AND MITIGATION STRATEGY PROGRAM: 3-YEAR SAFETY DATA ASSESSMENT**

Charles Dharmani, DrPH, PharmD, MPH, MS[1]; Charles Dharmani, DrPH, Pharm D, MPH, MS[2]; Maha Kandil, MD[1]; Maura Fallon, MD[3]; Margaret Wooddell, PhD, MBA[1]; Maribel Salas, MD, MSc, DSc[1], (1) Daiichi Sankyo, Inc., Basking Ridge, NJ, (2) Daiichi Sankyo Inc., Basking Ridge, NJ, (3) Daiichi Sankyo UK Ltd., Uxbridge, England, United Kingdom

Objective: Pexidartinib is a first-in-class colony-stimulating factor-1 receptor blocker indicated for the treatment of adult patients with symptomatic tenosynovial giant cell tumor (TGCT) associated with severe morbidity or functional limitations that are not amenable to improvement with surgery. Due to the potential risk of hepatotoxicity, pexidartinib (400 mg orally twice daily) was granted approval by the US Food and Drug Administration (FDA) on the condition of establishing a Risk Evaluation and Mitigation Strategy (REMS) program. The 3-year (August 2019-June 2022) cumulative safety data collected from patients enrolled in the pexidartinib REMS (pREMS) program are described here.

Methods: The pREMS program consists of elements to ensure safe use, a communication plan, an implementation system, and a timetable for REMS assessments. Healthcare providers prescribing pexidartinib must be both educated and certified in the pREMS program and educate their patients with TGCT who are receiving the drug. Dispensing pharmacies and distributors of pexidartinib must also be certified in the program. Scheduled safety assessments were collected on Patient Access Forms (PAFs).

Results: During the 3-year period, 451 TGCT patients, 369 prescribers, 2 wholesalers/distributors, and 2 pharmacies were certified or enrolled in the pREMS program. Of

the adverse events (AEs), the majority (94.2%) were nonserious. Hair color changes were the most frequently reported nonserious AE (4.4%), and increased aspartate aminotransferase (AST; 0.5%) was the most frequently reported serious AE. Of the 451 TGCT patients, only 51 (2.4%) lab tests had ALT or AST liver enzymes >3× ULN and TBIL >2× ULN, 17 (0.8%) had ALT or AST >10× ULN with/without TBIL elevation, and 12 (0.6%) had TBIL ≥2× ULN without changes in ALT or AST. Twenty-one cases (4.7%) that met the criteria for a liver AE indicated that the pattern of reported hepatic injury was consistent with the ENLIVEN trial results. There were no events of irreversible liver injury. Following initiation of pexidartinib, treatment interruption was seen in 98 (21.7%) patients, which was (n=106) primarily due to hepatic AEs (n=24; 22.6%), nonhepatic AEs (n=15; 14.2%), disease progression (n=2; 1.9%), and death (n=1; 0.9%). In patients resuming treatment, all were at a lower dose. Treatment discontinuation was reported in 132 patients (29.3%; 137 events), primarily due to hepatic AEs (n=26; 19%), nonhepatic AEs (n=23; 16.8%), disease progression (n=13; 9.5%), confirmed pregnancy (n=2; 1.5%), and death (n=1; 0.7%; not related to hepatotoxicity). Based on Liver AE Report Forms (LAERFs; 65 patients, 95 LAERFs), 12 (12.6%) cases required hepatology referral, 14 (14.7%) liver ultrasound, 12 (12.6%) other liver imaging, and 5 (5.3%) endoscopic retrograde cholangiopancreatography. Of these, 14 (14.7%) cases required hospitalization, 1 (1.1%) liver dialysis, 6 (6.3%) medication prescribed to treat liver injury, and 7 (7.4%) “other.” Liver AEs resolved in 33 (34.7%) cases, 51 (53.7%) cases were ongoing, and 1 (1.1%) case resolved with sequelae. No liver transplant or hepatotoxicity-related death was reported.

Conclusion: The safety profile of pexidartinib remains consistent with the data from the ENLIVEN trial. Furthermore, no new hepatic safety signals were identified. Based on data collected from the pREMS program, careful monitoring of liver enzyme levels combined with early intervention using dose modifications and permanent discontinuation are being actively conducted to mitigate the risk of potential hepatotoxicity. Overall, the results of this cumulative safety data assessment suggest that the pREMS program is ensuring the safe use of pexidartinib in patients with TGCT.

(P 200) EPIDEMIOLOGY OF TENOSYNOVIAL GIANT CELL TUMOR IN THE UNITED STATES

Charles Dharmani, DrPH, Pharm D, MPH, MS[1]; Charles Dharmani, DrPH, PharmD, MPH, MS[2], (1) Daiichi Sankyo Inc., Basking Ridge, NJ, (2) Daiichi Sankyo, Inc., Basking Ridge, NJ

Objective: Tenosynovial giant cell tumor (TGCT) is a rare non-malignant neoplasm involving the synovium and tendon sheaths that typically presents in young and middle-aged adults.1 TGCT is usually a monoarticular disease that involves the bone, soft tissue, synovium, or tendon sheath of small or large joints. Initial symptoms are often minimal due to the slow progressive nature of the disease, but as the tumor mass grows within the intra articular space and surrounding tissue, symptoms can progress from mild to debilitating. Commonly reported symptoms include pain, stiffness, swelling, and reduced range of motion of the affected joint, which can become severe and result in marked functional limitation.2 Localized TGCT (L-TGCT) is usually a benign neoplasm, most commonly occurring in the digits.3 Diffuse TGCT (D-TGCT) is a locally aggressive, nonmalignant neoplasm composed of synovial-like mononuclear cells, multinucleate giant cells, foam cells, siderophages, and inflammatory cells which may be intra-articular or extra-articular. D-TGCT most commonly occurs in large joints, particularly the knee as well as the ankle and hip.4 There is paucity of published scientific

literature on the epidemiology of TGCT. Only two population-based studies, one from the Netherlands (Mastboom et al., 2017) and the other from Denmark (Ehrenstein et al., 2017) have reported TGCT incidence rate estimates.1,3 Moreover, the single study (Myers and Masi, 1980), estimating the incidence of TGCT in the US is based on one US-county.5 No studies have been conducted that provide the population-based estimates of TGCT incidence and prevalence in the United States (US). The goal of this research is to estimate the overall and gender-specific annual incidence and prevalence of L-TGCT and D-TGCT in the US.

Methods: To estimate the burden of both L-TGCT and D-TGCT in the US, overall and gender-specific TGCT incidence rates reported by Mastboom et al. (2017) in the Netherlands1 were extrapolated to the age- and gender-specific US population based on 20206 census estimates. Overall and gender-specific prevalence reported by Ehrenstein et al. (2017) in Denmark3 were extrapolated to the overall and gender-specific adult (≥18 years) US population census estimates for 20206 to calculate the prevalence of TGCT in the US.

Results: The estimated overall annual incidence of L-TGCT of digits was 11,059 (Females=6,632 and Males=4,305), 3,578 new cases of L-TGCT of extremities (Females=2,155 and Males=1,435), and 1,626 new cases of D-TGCT (Females=995 and Males=638). This corresponds to an overall annual incidence 16,263 of TGCT in the US in 2020 (Localized=14,637; Diffuse=1,626). The estimated adult prevalence of L-TGCT was 144,094 (Females=88,044 and Males=56,290) and D-TGCT is 37,405 of (Females=20,063 and Males=17,541). This yields an overall estimated prevalence of 181,499 of adult TGCT in the US in 2020.

Conclusion: These results suggest that in the United States, the estimated prevalence of L-TGCT and D-TGCT are 144,094 and 37,405 respectively. Thus, there exists an estimated prevalence of 181,499 adult TGCT patients in the US. These estimates reveal that a large population of TGCT patients exists in the United States with a significant unmet need. Surgery, repeat surgery due to recurrence, and pexidartinib are the only treatment options available for these patients. In the United States, pexidartinib is the only drug approved by the Food and Drug Administration for the treatment of adult patients with symptomatic TGCT associated with severe morbidity or functional limitations and not amenable to improvement with surgery. Future efforts are needed to identify and develop additional treatment options for this underserved patient population.

(P 201) PLEOMORPHIC RHABDOMYOSARCOMAS: A NEW ENTITY ASSOCIATED WITH LYNCH SYNDROME? FIRST RESULTS OF SARCLYNCH NATIONAL STUDY

François Poumeaud, MD[1]; Nadim Fares, MD, PhD[2]; Pierre Vande Perre, MD[3]; Camille Tlemsani, MD, PhD[4]; Nicolas Penel, MD,PhD[5]; Benjamin Verret, MD[6]; Anabelle Sabouret, MD[6]; Sarah Watson, MD, PhD[7]; Emmanuelle Fourme, MD, PhD[8]; Sylvie Bonvalot, MD, PhD[7]; edouard Cottereau, MD[9]; Anne Ducassou, MD[10]; Dimitry Gangloff, MD[11]; Thomas Meresse, MD[11]; Jean-Christophe Thery, MD[12]; Marie Coudert, MD[13]; Allan Lancon, MD[14]; Estelle Cauchin, MD[15]; Gaëlle Perot, PhD[16]; Sophie Lejeune, MD[17]; Bertille Segier, Master[11]; Jean-Yves Blay, MD, PhD[18]; Philippe Rochaix, MDPhD[3]; Anne Gomez-Mascard, MD, PhD[19]; Marion Jaffrelot, MD[11]; Frederic Chibon, PhD[16]; Janick selves, MD, PhD[11]; Rosine Guimbaud, MD,

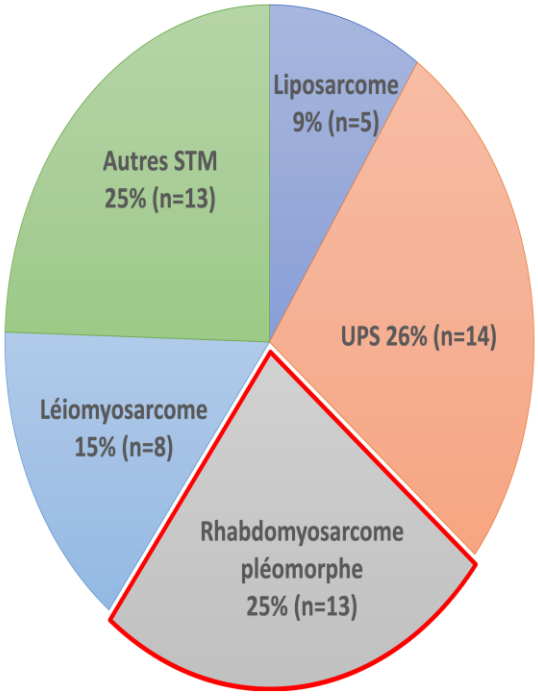
PhD[2]; Thibaud Valentin, MD[3], (1) Oncopole Claudius Regaud, Toulouse, Languedoc-Roussillon, France, (2) CHU Toulouse, Toulouse, Midi-Pyrenees, France, (3) Oncopole Claudius Regaud, Toulouse, Midi-Pyrenees, France, (4) APHP, Paris, Ile-de-France, France, (5) Centre Oscar Lambret, Lille, Nord-Pas-de-Calais, France, (6) Gustave Roussy, Paris, Ile-de-France, France, (7) Institut Curie, Paris, Ile-de-France, France, (8) Curie, Paris, Ile-de-France, France, (9) CHU Tours, Tours, Pays de la Loire, France, (10) OCR, TOULOUSE, Midi-Pyrenees, France, (11) OCR, Toulouse, Midi-Pyrenees, France, (12) CHU Rouen, Rouen, Bretagne, France, (13) ICP, Angers, Pays de la Loire, France, (14) CGF, Dijon, Champagne-Ardenne, France, (15) ICO, Nantes, Pays de la Loire, France, (16) Cancer Research Center of Toulouse, Toulouse, Midi-Pyrenees, France, (17) CHU Lille, Lille, Nord-Pas-de-Calais, France, (18) Centre Léon-Bérard, Lyon, Rhone-Alpes, France, (19) Institut Universitaire du Cancer de Toulouse, Toulouse, Midi-Pyrenees, France

Objective: Historically, sarcomas do not belong to Lynch Syndrome (LS) spectrum. However, we recently published review of literature (Poumeaud et al, Current Opinion in Oncology 2023) reporting unusual high proportion of pleomorphic rhabdomyosarcomas (pRMS) in Lynch patients having developed a sarcoma (10%). The main objective of SarcLynch is to verify these data in real life through GSF-GETO cohort.

Methods: SarcLynch is a French national multicentric retro-prospective cohort describing clinical and tumoral characteristics of sarcomas developed in LS patients. Patients were identified through Netsarc and oncogenetic OfeLy databases. All patients with a proven LS having developed a bone sarcoma (BS) or a soft-tissue sarcoma (STS) were included. Patients with a syndromic variation of LS or Lynch-like syndrome were excluded. All cases were reviewed in a sarcoma expert center.

Results:Eighty-five patients were pre-identified. We present the results obtained on the first 78 patients, included from 15 centers in France. Median age at diagnosis was 51 years old (15-85 y.o). Among patients with available data, 52 (72%) have a personal history of cancer (whom 50 belonging to LS spectrum). Sixty-five patients (83.3%) developed a STS while 13 developed a BS (16.7%). Among STS, undifferentiated pleomorphic sarcomas (UPS, n=18, 27.7%) and pRMS (n=14, 21.5%) are most diagnosed sub-types. Chondrosarcomas (n=6, 46.2%) are most represented BS. Most patients (n=31, 39.8%) present a germinal mutation of MSH2 gene. Mismatch repair somatic phenotype is available for 36 patients in immunohistochemistry (IHC) with 24 patients being deficient mismatch repair (dMMR, 66.7%). Microsatellite instability assessed by Pentaplex-PCR is available for 28 patients, whom 13 are micro-satellite instable (MSI). Eight patients (30.4%) exhibit discrepancy between IHC and PCR assessment. Seven patients (9%) were treated with immune checkpoint inhibitors (ICI, 6 with pembrolizumab and 1 with nivolumab). Six patients responded positively with stable or partial responses. Duration of response varies from 6 to 28 months in patients with significant CPS and tumoral mutational burden (TMB) scores.

Conclusion: These results are consistent with those previously published in literature, with an unusually high proportion of pRMS in LS patients (21.5% vs < 1% in sporadic STS) and in patients with germline mutation of MSH2 (39.8% vs 25% in LS patients). Despite the few patients treated with ICI (n=7, 9%), most of them (n=6, 85.7%) favorably responded to anti-PD1/PD-L1 with some extended responses. Similarly, biomarkers predictive of response to ICI (CPS, TMB) seem to be increased, consistently with LS-carcinomas. First results of SarcLynch report an unusually high proportion of PRM in LS patients, suggesting a possible interest to screen for LS in PRM. A better comprehension of genomic instability in this subgroup is essential and will be performed in SarcLynch-GI study.



	Total
	(n = 62)
Sexe (n=62)	
Men	29 (46.7%)
Women	33 (53.3%)
Localization (n=59)	
Members	37 (62.7%)
Trunc	7 (11.9%)
Abdomen	13 (22%)
Head and Neck	2 (3.4%)
Stage at diagnosis (n=56)	
Localized	50 (89.3%)
Métastatic	6 (10.7%)

	Total
Age at diagnosis (Y) (n=61)	
Median (Extremes)	**52.6 (15.0;85.0)**
Personal history of cancer(n=59)	
No	25 (42.3%)
Yes	**34 (57.6%)**
Including CCR	**33 (97.1%)**
Mutated MMR gene (n=62)	
MLH1	17 (27.4%)
MSH2	**28 (45.2%)**
MSH6	15 (24.2%)
PMS2	2 (3.2%)

(P 202) INFLAMMATORY MYOFIBROBLASTIC TUMOUR WITH ROS1 GENE FUSIONS IN CHILDREN AND YOUNG ADOLESCENTS

Reineke A. Schoot, MD, PhD[1]; Daniel Orbach, MD[2]; Veronique Minard Colin, Prof. dr.[3]; Rita Alaggio, MD[4]; Daniela Di Carlo, MD[5]; Nadege Corradini, MD[6]; Federico Mercolini, MD[7]; Giuseppe M. Milano, MD, PhD[8]; Max M. van Noesel, MD, PhD[1]; Angelique Rome, MD[9]; Patrizia Dall'Igna, MD, PhD[10]; Kristian Pajtler, MD[11]; Monika Sparber-Sauer, MD[12]; Andrea Ferrari, MD[13]; Michela Casanova, MD[13], (1) Princess Máxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands, (2) SIREDO Oncology Center (Care, Innovation and Research for Children and AYA with Cancer), Institut Curie, and University PSL, Paris, Ile-de-France, France, (3) Gustave-Roussy, Université Paris-Saclay, Villejuif, Ile-de-France, France, (4) Bambino Gesu Children's Hospital, IRCCS, Rome, Lazio, Italy, (5) University Hospital of Padova, Padova, Veneto, Italy, (6) Institut d'Hematologie et d'Oncologie Pédiatrique, Centre Léon Bérard, Lyon, Rhone-Alpes, France, (7) "Lalla Seràgnoli", Istituto di Ricovero e Cura a Carattere Scientifico, Azienda Ospedaliero-Universitaria di Bologna,, Bologna, Emilia-Romagna, Italy, (8) Bambino Gesù Children's Hospital, Istituto di Ricovero e Cura a Carattere Scientifico (IRCSS), Rome, Lazio, Italy, (9) Timone Children's Hospital, Marseille, Provence-Alpes-Cote d'Azur, France, (10) Department of Precision and Regenerative Medicine and Jonic Area,Pediatric Hospital Giovanni XXIII, University of Bari, bari, Puglia, Italy, (11) Hopp Children’s Cancer Center Heidelberg (KiTZ), Heidelberg University, Heidelberg, Baden-Wurttemberg, Germany, (12) Zentrum für Kinder-, Jugend- und Frauenmedizin (Olgahospital), Stuttgart, Baden-Wurttemberg, Germany, (13) Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Lombardia, Italy

Objective: Inflammatory Myofibroblastic Tumours (IMTs) are very rare tumours that may occur in children, adolescents, and adults. Although IMTs are of intermediate malignancy, they may be locally aggressive and difficult to resect. IMTs are often driven by ALK fusions and less frequently by alternative fusions such as ROS1. As a result, targeted treatments (ALK/ROS1 inhibitors) have become available for these patients. We describe the clinical characteristics, treatment approach and outcome for a series of young patients with IMTs and ROS1 alterations.

Methods: Retrospective, international, multi-centre study analysing young patients (< 21 years) with ROS1 altered IMTs, treated in ten European referral centres between 2014 and 2022. Patients were included in the EpSSG NRSTS-2005 protocol or registered in the SoTiSaR registry. Primary surgery was recommended if a microscopic radical resection was feasible without mutilation. No standard systemic treatment protocol was available, but several medical options were recommended.

Results: A total of 19 patients (median age 8.3 years) were included. Most patients had a biopsy at diagnosis (IRS I; n=2, IRS II; n=1, IRS III biopsy; n=11, IRS III resection; n=3, IRS IV; n=2). Twelve patients received neo-adjuvant systemic therapy in first line (four received multiple treatments): high dose steroids (n=2), vinorelbine/vinblastine with methotrexate (n=6), or ROS1 inhibitors (n=8). After a median follow-up of 2.8 years (range, 0.2-13.4), seven patients developed an event. Two patients developed loco-regional progression, three had a loco-regional relapse, one developed both progression of the primary tumour and of a metastatic lesion in the central nervous system, and one patient had persistent disease with systemic symptoms. The 3-year event free survival was 41% (95% confidence interval 11-71%) and 3-year overall survival 100%.

Conclusion: Outcome for ROS1 altered IMTs appears excellent. A complete resection at diagnosis was often considered not feasible and most patients needed neo-adjuvant therapy. Patients that developed a tumour event could be cured with re-initiation of systemic therapy and/or surgery. This approach illustrates a switch in treatment philosophy moving from immediate, often mutilating, surgery to systemic (targeted) therapy as a bridge to more conservative surgery later in the treatment course.

(P 203) PRECLINICAL EVALUATION AMG 650, A SMALL MOLECULE INHIBITOR OF KIF18A, IN PEDIATRIC SOLID TUMORS BY THE PEDIATRIC PRECLINICAL IN VIVO TESTING (PIVOT) PROGRAM

Wendong Zhang, BS[1]; Richard Gorlick, MD[1]; Michael Roth, MD[2]; Jonathan Gill, MD[1]; Yifei Wang, MD[2]; Zhongting Zhang, MS[2]; Yizheng Tu, MD[2]; E. Anders Kolb, MD[3]; Carol J Bult, PhD[4]; Emily L Jocoy, PhD[4]; Raushan Kurmasheva, PhD[5]; Peter Houghton, PhD[5]; Beverly Teicher, PhD[6]; Malcolm A Smith, MD, PhD[7], (1) The University of Texas MD Anderson Cancer Center, Houston, TX, (2) UT MD Anderson Cancer Center, Houston, TX, (3) 2Nemours Center for Cancer and Blood Disorders, Wilmington, DE, (4) The Jackson Laboratory, Bar Harbor, ME, (5) Greehey Children's Cancer Research Institute, San Antonio, TX, (6) National Cancer Inst., Bethesda, MD, (7) National Cancer Inst., Rockville, MD

Objective: KIF18A is a mitotic kinesin that plays a role in maintaining bipolar spindle integrity during mitosis. KIF18A knockdown selectively inhibits growth and induces apoptosis for cancer cell lines with chromosomal instability (CIN). AMG 650 is a selective inhibitor of KIF18A that phenocopies the effects observed for KIF18A knockdown. Osteosarcoma is characterized by a chaotic genome with chromothripsis and a large number of chromosomal structural variants. Anaplastic Wilms tumor is associated with TP53 mutation as are some Ewing sarcoma cases, and alveolar rhabdomyosarcoma often exhibits tetraploidy. In this study the pre-clinical efficacy of AMG 650 was tested using PDX models for these cancers.

Methods: AMG 650 was tested in 7 osteosarcoma, 2 Wilms tumor, 3 rhabdoid tumor, 15 rhabdomyosarcoma, and 7 Ewing sarcoma PDX models. A conventional testing design was used for osteosarcoma models with ten AMG 650-treated and ten vehicle control animals for each model. A single mouse trial design was used for the other cancer types, with a single vehicle control and AMG 650-treated mouse for each model. AMG 650 was administered orally at the dose of 100 mg/kg for 21 days. Mice were monitored for 12 weeks. Objective response (partial and complete) was assessed based on serial tumor volume measurements, and for osteosarcoma models median time to event (event defined as 4-fold increase in tumor volume) was evaluated using previously described PIVOT program testing methods. Plasma was collected for pharmacokinetic studies at different time points following the final administered dose (2h, 4h, 6h, 12h, and 24h; n=2 per time point).

Results: For 6 evaluable osteosarcoma models, no objective responses were observed, and a single model showed > 2-fold increase in median time to event. Among 15 rhabdomyosarcoma models, one achieved a complete response with tumor regrowth beginning approximately 3 weeks after treatment completion. Among 8 Ewing sarcoma models studied, 2 achieved partial responses with tumor regrowth occurring on-treatment or within several weeks of treatment completion. No objective responses were observed for the 2 Wilms tumor models and 3 rhabdoid tumor models tested. Drug levels were in line with previous results and will be presented.

Conclusion: In osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma models, antitumor activity of AMG 650 was variable, ranging from no objective responses to one model with a complete response. Identification of specific determinants of sensitivity and the evaluation of activity in combination will aid in potential use of AMG 650 in these pediatric tumor settings.

(P 204) RADIATION-INDUCED ANGIOSARCOMA OF THE BREAST: RETROSPECTIVE ANALYSIS AT A REGIONAL TREATMENT CENTRE

Han H. Wong, PhD FRCP[1]; Elena Cojocaru[1]; James Watkins, PhD FRCPath[1]; Sujil James, n/a[2]; Tony Aloysius, n/a[2]; Jennifer Harrington, MA MB BChir MRCP PhD[1]; Gail Horan, n/a[1]; Helen Hatcher, n/a[1], (1) Cambridge University Hospitals NHS Foundation Trust, Cambridge, England, United Kingdom, (2) University of Cambridge, Cambridge, England, United Kingdom

Objective: Radiation-induced angiosarcoma (RIA) of the breast is an uncommon but morbid complication after radiotherapy for breast cancer. This retrospective study analysed the treatment and outcome of breast RIA patients at Cambridge University Hospital (CUH), a regional treatment centre in the East of England.

Methods: We retrospectively identified all patients diagnosed with and treated for breast RIA

between 2010 and 2022, at CUH as well as at four other regional district hospitals who were referred to us. All patients were included in the data analysis.

Results: 22 patients were identified. The median age of RIA diagnosis was 65 years (range 41-78). The median time from completion of breast radiotherapy to RIA diagnosis was 6.5 years (range 2.4-16.0) – this interval appears to have decreased over the last 24 years (r2 = 0.6601). Tumours were often multifocal, with a median size of 65 mm (range 10-250). Two (9%) patients had metastasis at presentation. All patients underwent surgery (55% at CUH, 45% at local hospitals). Six (27%) patients received peri-operative pegylated liposomal doxorubicin (PLD) in the first-line setting. 13 (62%) patients relapsed following their primary curative-intent treatments after a median of 28 months – 10 with locoregional disease and three with distant metastases. Three patients with local recurrence received peri-operative weekly paclitaxel. After a median follow-up period of 66.5 months, the median progression-free survival (PFS) following primary curative-intent surgery was 28 months (2-year and 5-year PFS rates of 55% and 33%, respectively). The median overall survival (OS) was 82 months (2-year and 5-year OS rates of 73% and 60%, respectively). Metastases occurred in eight (36%) of our patients, the commonest sites being lung (100%) and lymph nodes (50%), followed by bone and liver. Analysis of potential prognostic variables were performed: age (≤72 vs >72 years), tumour size (< 5 vs ≥5 cm), surgical margin (≤1 mm vs >1 mm), hospital where the primary surgery was performed (CUH vs other hospitals), the use of peri-operative chemotherapy (vs surgery only), and peri-operative chemotherapy regimen (PLD vs paclitaxel). No factors other than age were found to be statistically significant in affecting PFS and OS. Patients of age >72 years have statistically shorter median PFS following primary surgery (6 vs 29 months; HR = 4.586 (95% CI 1.184-17.76); logrank P = 0.0275) as well as OS (45 vs 102 months; HR = 7.129 (95% CI 1.646-30.88); logrank P = 0.0086) than those who were ≤72 years.

Conclusion: RIA is an aggressive disease with high rates of recurrence, morbidity and mortality, and appears to be occurring sooner after breast radiotherapy. It is clear that more effective and personalised systemic therapies, selected or developed through in-depth understanding of the molecular and genetic basis of RIA, are desperately needed to improve the outcome of this group of patients. The current availability and provision of whole-genome sequencing for sarcoma patients in the UK could help to achieve this.

(P 205) PRIMARY CARDIAC SARCOMA: A RETROSPECTIVE ANALYSIS OF PRESENTATION, MANAGEMENT AND OUTCOMES

Luke Smith[1]; Han H. Wong, PhD FRCP[1]; Gill Gray[2]; Helen Hatcher, n/a[1], (1) Cambridge University Hospitals NHS Foundation Trust, Cambridge, England, United Kingdom, (2) Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, England, United Kingdom

Objective: Primary cardiac sarcoma is exceedingly rare and has a poor prognosis. This highly aggressive malignancy leads to progressive heart failure and death without treatment. Here, we review the presentation, management and outcomes of these patients at a regional sarcoma treatment centre and its associated cardiothoracic hospital.

Methods: Medical records of patients diagnosed with and treated for cardiac sarcoma at Addenbrooke's Hospital and the Royal Papworth Hospital in Cambridge, UK, between 2011 and 2022, were reviewed. All patients were included in the data analysis.

Results: 11 patients were diagnosed with cardiac sarcoma (6 males, 5 females); median age of presentation was 31 years (range 19-73). Presenting features included breathlessness (6), cough (4), chest pain (2), peripheral oedema (1), constitutional symptoms (1), haemoptysis (1) and transient ischaemic attack (1). Cardiac function at diagnosis was often preserved: 5 had normal left ventricular ejection fraction (≥55%), 3 had moderate impairment (30-55%), and 1 with < 30% in the context of previous intensive treatment for childhood leukaemia. Sites of disease were: atrium (8), ventricle (2), and mass encasing the heart (1). Over half (55%) had evidence of metastasis at presentation. Histological findings were angiosarcoma (6), intimal sarcoma (2), high-grade sarcoma NOS (1) and synovial sarcoma (1). The median tumour dimension was 62 mm (range 27-135). Five patients underwent excision of the primary tumour: 2 with curative intent, 2 debulking surgeries, and 1 had surgery for what was thought to be atrial myxoma. One did not have biopsy/surgery due to significant co-morbidities. Adjuvant ifosfamide chemotherapy was administered after excision of intimal sarcoma (2) and synovial sarcoma (1). In the palliative setting, partial responses were observed in angiosarcoma with paclitaxel, anthracyclines, gemcitabine and docetaxel, and in synovial sarcoma with ifosfamide. Two patients received the checkpoint inhibitors ipilimumab and nivolumab but neither obtained clinical benefit. The median overall survival (OS) of all patients was 19.5 months (2-year OS rate of 30%). OS was significantly worse in patients with advanced/metastatic disease (vs localised resectable tumour; HR=7.86 (95% CI 1.604-38.5), P=0.011) and primary tumour size of ≥90 mm (HR=36.43 (95% CI 2.99-443.8), P=0.0048). There were only 2 long term surviving patients (76 and 99 months, respectively) – both had R1 resection of atrial intimal sarcoma followed by adjuvant ifosfamide chemotherapy and radiotherapy.

Conclusion: Cardiac sarcomas can present with non-specific symptoms and may be initially misdiagnosed as other benign entities. Unsurprisingly, outcome is significant better for patients with smaller or localised and resectable tumour than those with advanced or metastatic disease. The use of adjuvant ifosfamide chemotherapy (with close monitoring of fluid balance) and radiotherapy following R1 resection of intimal sarcoma appeared to be associated with long-term survival. Further study is required to elucidate how to manage this rare tumour in the adjuvant and palliative settings.

(P 206) A SINGLE-ARM, PHASE II STUDY OF NEOADJUVANT PEMBROLIZUMAB COMBINED WITH RADIOTHERAPY IN THE PRIMARY TREATMENT OF SOFT TISSUE SARCOMAS.

Lee D. Cranmer, MD, PhD, F.A.C.P.[1]; Elizabeth T. T. Loggers, MD, PhD[2]; Michael J. Wagner, MD[3]; Edward Kim, MD[4]; Stephanie K. Schaub, MD[4]; Gabrielle M. Kane, MB EdD FRCPC[4]; Jesse Roberts, M.D.[4]; Matthew J. Thompson, MD[5]; Harveshp Mogal, MD[4]; Jeremy Sharib, MD[4]; Robert W. Ricciotti, MD[4]; Eleanor Chen, MD, PhD[4]; Jose G. Mantilla, MD[6]; Anshu Bandhlish, MD[4]; Roxanne Moore, MS[4]; Ralph G. Black, B.S. General Biology[7]; Niall Morin, n/a[4]; Shannon Maxwell[4]; Rylee Johnson, n/a[4]; Seth Pollack, MD[8], (1) University of Washington/Fred Hutchinson Cancer Center, Seattle, WA, (2) Fred Hutch Cancer Center, Seattle, WA, (3) University of Washington/Fred Hutch/Seattle Cancer Care Alliance, Seattle, WA, (4) University of Washington, Seattle, WA, (5) Desert Orthopedics, Bend, OR, (6) NYU Langone Health, New York, NY, (7) Fred Hutchinson Research Center, SEATTLE, WA, (8) Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL

Objective: Relapse after definitive surgery and radiotherapy (RT) is a common problem in STS. Past evidence has suggested potentially beneficial immunotherapeutic effects of RT. Immunotherapy with Programmed Death-1 receptor (PD-1) inhibitors has demonstrated preliminary or confirmed activity in a number of types of sarcomas, including undifferentiated pleomorphic sarcomas, cutaneous angiosarcomas, and alveolar soft part sarcomas. Combined therapy with PD-1 inhibitors and RT may yield enhanced efficacy versus either modality alone. Here, we investigated the feasibility and clinic-pathologic outcomes of concurrent therapy with the PD-1 inhibitor pembrolizumab (PEM) and neoadjuvant RT.

Methods: Patients ≥18 years were eligible if they had localized STS for which both surgical resection and neoadjuvant RT to 50-50.4 Gy (institutional standard) were determined to be feasible and appropriate by experienced sarcoma practitioners. Patients with FNCLCC grade 3 (>1.5 cm) or grade 2 (>3 cm) of any location were eligible. Bone sarcomas and STS for which chemotherapy is routinely administered at our institution were excluded. Patients with metastatic disease at baseline or contraindications to PD-1 inhibitor therapy were excluded. All patients received 3 doses of PEM (200 mg IV q21 d) concurrently with planned neoadjuvant RT. 4-6 weeks after neoadjuvant therapy, patients underwent planned surgical resection. The primary endpoint was the proportion of patients with complete pathologic necrosis, defined as ≥95% pathologic necrosis at the time of definitive surgical resection. Enrollment of 26 subjects has 94.4% power to detect an improvement in the complete necrosis rate from 15% to 40%. The observation of at least 7 subjects with ≥95% pathologic necrosis would be considered evidence to rule out a 15% complete necrosis rate. Secondary endpoints included extent of surgical resection achieved; adverse events (AE) by CTCAE 5.0; local and distant progression-/relapse-free survival (PFS/RFS) from time-of-PEM initiation and from time-of-surgery. Prior to surgery, only confirmed development of distant metastases, local progression preventing surgical resection, or death from any cause were considered to represent events from the perspective of PFS/RFS. After surgery, any documented progression, or death from any cause, were considered events for purposes of RFS endpoints. Survival endpoints were analyzed by the Kaplan-Meier method. Disease response and progression were defined according to RECIST 1.1 criteria.

Results: 26 patients were evaluable; 21 (81%) had trunk/extremity and 5 (19%) had retroperitoneal/visceral disease. Histologies included undifferentiated pleomorphic sarcoma (UPS; 9/35%), dedifferentiated liposarcoma (8/31%), myxofibrosarcoma (2/8%), solitary fibrous tumor (2/8%), and other sarcoma (5/19%). 24 (92%) received all three planned PEM doses; 2 (8%) received only 2 doses (G2 rash, G2 fatigue). All completed planned RT. Two UPS patients had objective partial responses at pre-operative imaging (ORR=8%). Progressive disease prior to surgery occurred in 3: 2 (8%) with local progression only and 1 (4%) with metastatic pulmonary disease. All three proceeded with surgery. All remaining patients had stable disease. Median pathologic primary tumor size was 10 cm (range 2.1-26 cm). 14 (54%) patients had confirmed FNCLCC grade 3 tumors at baseline, while the remaining 12 (46%) were ≥grade 2. Negative margins were achieved in 23/26 (88%). Median follow-up time at data cut-off (19 June 2023) was 18.8 months (m) from PEM initiation (PI) (range 2.0-59.2 m). At that time, 3 patients had no post-operative imaging and 4 patients had died: sarcoma progression (2), co-morbidity (1), ischemic colitis (1). Median pathologic necrosis was 33% (range 0-99%; n=24), with 4 (17%) demonstrating ≥95% pathologic necrosis, the primary endpoint. Median percent viable cells was 25% (range 1-100; n=21), with 6 (29%) demonstrating less than 10% viable. There were 11 PFS/RFS events in the evaluable population from PI and 10 RFS events from surgery. Median PFS/RFS of all evaluable PEM-treated patients from PI was 17.2 m (95% CI 7.6-NR m). Median RFS from surgery for those without metastatic disease pre-operatively and at least one post-operative imaging assessment was 14.6 m (95% CI 5.1-NR m). All relapses were distant. There were no grade 5 AE’s judged to be related to study therapy. AE’s were

manageable and consistent with the anticipated effects of PEM and RT.

Conclusion: Neoadjuvant therapy combining PEM and RT is feasible in the management of STS, including in retroperitoneal sarcomas. AEs were consistent with known PEM and RT effects, without evidence of synergism. This study did not achieve its primary endpoint, with only 4 patients demonstrating ≥95% pathologic necrosis. Objective responses were documented pre-operatively in two patients, both with UPS. Updated outcomes analyses and preliminary results of correlative studies will be presented.

(P 207) A MULTICENTER PHASE II STUDY OF CABOZANTINIB PLUS NIVOLUMAB FOR PATIENTS WITH ADVANCED ANGIOSARCOMA PREVIOUSLY TREATED WITH A TAXANE: ALLIANCE A091902

Juneko Grilley-Olson, MD[1]; Jacob Allred, MS[2]; Scott M. Schuetze, MD, PhD[3]; Elizabeth J. Davis, MD[4]; Andrew Poklepovic, MD[5]; Michael J. Wagner, MD[6]; Richard F. Riedel, MD[7]; Meng Xu-Welliver, MD, PhD[2]; Stephanie Berg, DO[8]; Suzanne George, MD[9]; Steven Robinson, MBBS[2]; Paul Googe, MD[10]; Sandra P. D'Angelo, MD[11]; Gary K. Schwartz, MD[12], (1) Duke University, Durham, NC, (2) Mayo Clinic, Rochester, MN, (3) University of Michigan, Ann Arbor, MI, (4) Vanderbilt University Medical Center, Nashville, TN, (5) Virginia Commonwealth University, Richmond, VA, (6) University of Washington/Fred Hutch/Seattle Cancer Care Alliance, Seattle, WA, (7) Duke Cancer Institute, Durham, NC, (8) Dana Farber Cancer Institute, Needham, MA, (9) Dana-Farber Cancer Institute, Boston, MA, (10) University of North Carolina, Chapel Hill, NC, (11) Memorial Sloan Kettering Cancer Center, New York, NY, (12) Herbert Irving Comprehensive Cancer Center at Columbia University School of Medicine, New York, NY

Objective: Collectively, sarcomas have demonstrated less benefit from immunotherapy than many other cancers. Pts with AS have limited options, particularly after taxane chemotherapy. Although AS is a vascular malignancy, anti-VEGF therapies have minimal monotherapy clinical activity. Some cutaneous AS have a high UV damage signature, but other AS often do not. Cabozantinib (C) is a multikinase inhibitor that may alter PD-1 expression in regulatory T cells, promoting an immune permissive environment when combined with nivolumab (N), a fully human anti PD-1 MAB. We hypothesize that the combination of C+N will be an effective therapy in AS, across AS subtypes.Collectively, sarcomas have demonstrated less benefit from immunotherapy than many other cancers. Pts with AS have limited options, particularly after taxane chemotherapy. Although AS is a vascular malignancy, anti-VEGF therapies have minimal monotherapy clinical activity. Some cutaneous AS have a high UV damage signature, but other AS often do not. Cabozantinib (C) is a multikinase inhibitor that may alter PD-1 expression in regulatory T cells, promoting an immune permissive environment when combined with nivolumab (N), a fully human anti PD-1 MAB. We hypothesize that the combination of C+N will be an effective therapy in AS, across AS subtypes.

Methods: This open-label multi-arm study enrolled pts with locally advanced/metastatic AS. Arm 3, reported here, enrolled pts who had received a taxane for AS (including adjuvant) prior to study enrollment. Pts were not restricted on number of prior lines, but prior anti-VEGF and immunotherapies were not allowed. Eligible pts received C (40 mg po daily) with N (480 mg IV every 4 wks). Treatment was permitted beyond progressive disease (PD) in the 1st 12 wks (4

wk confirmatory scan), but PD response was censored at 12 wks. The Simon 2-stage design (null and alternative overall response rate (ORR) were 10% and 35%, respectively) required ≥1 confirmed response in 9 pts in the 1st stage, and >4 of 18 pts (91.4% power, alpha 0.095) for the primary endpoint. Secondary endpoints were adverse events (AEs), progression free, overall survival (PFS, OS), and pt-reported outcomes (PRO).

Results: 22 eligible pts [median age 67 yrs (32-92), 11 female] received ≥1 dose of C+N. Primary disease sites: 13 cutaneous (scalp/face), 1 liver, 4 breast, 2 other. All pts received prior taxanes (11/22=50% as adjuvant therapy) and 5/22 (23%) had also received prior anthracycline (all relapsed AS). Primary endpoint: 13 of the first 18 evaluable patients (72%) experienced objective response (OR, 95%CI: 47%-90%). After a median follow-up of 11.2 mo, 13 of 22 pts achieved OR (11 partial response, 2 complete), for ORR 59% (95%CI: 36-79%). Responses were seen in pts with primary cutaneous disease 7/13 (54%) and noncutaneous disease 6/9 (67%). Median PFS was 9.6 mo (5.4-NR), and OS has not been reached. Off treatment reasons include: progressive disease (15), comorbid condition unrelated to AS or study (1); 6 pts remain on study. Grade (G) 3 hypertension (HTN) was the only possibly treatment related AE (TRAE) occurring in >10%. Two G 4/5 TRAE were reported: HTN and dyspnea. PRO results are being analyzed.

Conclusion: C + N demonstrated significant antitumor activity in taxane-pretreated AS and was well tolerated without new safety concerns. Activity of the combination was seen across AS subtypes. PRO and exploratory analyses will be presented. Support: U10CA180821, U10CA180882, U24 CA196171; U10CA180820; https://acknowledgments.alliancefound.org. U10CA180868; U10CA180888.

(P 208) LOW INCIDENCE OF CANCER THERAPY-RELATED CARDIAC DYSFUNCTION IN ADULT SARCOMA PATIENTS RECEIVING DOXORUBICIN AND UPFRONT DEXRAZOXANE

Kimberly Middleton, PharmD[1]; Sasha Watson, PharmD[2]; Jill Lykon, PharmD[1]; Roiner Ruiz, PharmD[1]; Emily Jonczak, MD[1]; Jonathan Trent, MD[1]; Gina D'Amato, MD[1], (1) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL, (2) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Boca Raton, FL

Objective: Anthracycline-based chemotherapy remains standard first-line treatment for many patients with bone or soft tissue sarcoma. However, clinical utility of anthracyclines may be limited by cancer therapy-related cardiac dysfunction (CTRCD), including cardiomyopathy with left ventricular (LV) dysfunction. The estimated incidence of CTRCD is 3-5% at a 400 mg/m2 cumulative doxorubicin dose. This increases to 7-26% after 550 mg/m2 and up to 48% after 700 mg/m2. Due to therapeutic benefit, sarcoma patients frequently receive beyond the recommended cumulative dose of 400-550 mg/m2. Current strategies to decrease cardiotoxicity include administering doxorubicin as a continuous infusion or in combination with dexrazoxane, a cardioprotective agent. Prophylactic dexrazoxane is currently approved by the United States Food and Drug Administration (FDA) for use with doxorubicin doses above 300 mg/m2 in breast cancer, while upfront use remains controversial due to theoretical concerns for reduced efficacy, added myelosuppression, and secondary malignancies. To date, strategies to decrease anthracycline induced cardiotoxicity vary between sarcoma centers. The objectives of

this study were to evaluate the rate of CTRCD and treatment outcomes with the combination of doxorubicin and dexrazoxane upfront for the treatment of adult sarcoma patients at our institution.

Methods: This retrospective cohort study was approved by the University of Miami Institutional Review Board (IRB) and included 87 patients who received at least 400 mg/m2 of doxorubicin with dexrazoxane for the treatment of sarcoma from January 2015 to December 2021. Of the 87 patients, 57 received 400-550 mg/m2, 21 received 551-700 mg/m2, and 9 received above 700 mg/m2. The primary outcome was the incidence of CTRCD, defined as LV ejection fraction (LVEF) decrease of at least 10% from baseline resulting in LVEF less than 50%. Secondary aims included progression free survival (PFS), hospitalization for cardiac-related events, cardiac-related death, and secondary malignancy.

Results: CTRCD developed in 4/87 patients (4.6%). All four of the patients with CTRCD received cumulative doxorubicin doses between 400-550 mg/m2. Interestingly, 0/30 patients who received doses greater than 550 mg/m2 developed CTRCD. Two patients (2%) developed symptomatic CTRCD and both patients had metastatic disease. The median cumulative doxorubicin dose was 467 mg/m2 (range 430-1001 mg/m2) and median doxorubicin dose per cycle was 75 mg/m2 (range 60-96).

Conclusion: These results suggest upfront concomitant use of dexrazoxane with doxorubicin may be associated with lower rates of CTRCD. From our data there is no evidence to support a maximum cumulative doxorubicin dose recommendation when given in combination with upfront dexrazoxane. Limitations include retrospective nature, limited sample size, and lack of control arm. Further analysis, including cost-analysis, is warranted to support this practice.

Baseline Characteristics (n = 87)	
Sex, n (%)	
Male	39 (45)
Female	48 (55)
Age, median years (range)	53 (21-83)
Race, n (%)	
Caucasian	69 (79)
African American	13 (15)
Asian	3 (4)
Not Reported	2 (3)
Ethnicity, n (%)	
Hispanic and/or Latinx	50 (57)
Non-Hispanic or Non-Latinx	37 (43)
Diagnosis, n (%)	
Soft tissue sarcoma	72 (83)
Bone sarcoma	15 (17)
Metastatic when receiving doxorubicin, n (%)	33 (38)
Baseline EF, median % (range)	60-65 (50-70)
Baseline GLS, median % (range)	-21.8 (-14.1 to -26.9)

EF: ejection fraction, GLS: global longitudinal strain

Treatment Characteristics (n = 87)	
Cumulative doxorubicin dose, median mg/m^2 (range)	467 (430-1001)
Doxorubicin dose per cycle, median mg/m^2 (range)	75 (60-96)
Received radiation to cardiac field, n (%)	8 (0.9)
Received pazopanib, n (%)	26 (30)
Received trabectedin, n (%)	7 (8)
≥1 pre-existing cardiovascular risk factor*, n (%)	56 (64)
Concomitant cardioprotective medications†, n (%)	40 (46)

*smoking, diabetes mellitus, obesity, hypertension, hypercholesterolemia
†antihypertensives, statins, antiplatelets

Primary Endpoint	Outcome (n = 87)
CTRCD, n (%)	4 (4.6)
400-550 mg/m^2, n	4/57
551-700 mg/m^2, n	0/21
>700 mg/m^2, n	0/9
Symptomatic CTRCD, n (%)	2 (2.3)

Patient	Risk Factors/Characteristics
1 83 YOF	• Stage IV • Total cumulative dose: 445 mg/m^2 • LVEF decreased from 60-65% to 45-50% within two years • Received subsequent therapy with both trabectedin and pazopanib
2 60 YOF	• Stage IV • Total cumulative dose: 453 mg/m^2 • LVEF decreased from 50-55% to 40-45% within two years • Received pazopanib prior to doxorubicin and subsequent trabectedin • Previous smoker
3 54 YOM	• Stage IIIA • Total cumulative dose: 520 mg/m^2 • LVEF decreased from 55-60% to 40-45% within one year • Received radiation to the chest • Previous smoker
4 73 YOM	• Stage IV • Total cumulative dose: 520 mg/m^2 • LVEF decreased from 55-60% to 40-45% within one year • Received subsequent trabectedin • History of type 2 diabetes and previous smoker

YOF: year-old female, YOM: year-old male

(P 209) PROGNOSTIC VALUE OF EARLY RADIOLOGIC TUMOUR VOLUME RESPONSE IN RHABDOMYOSARCOMA; RESULTS OF THE EUROPEAN PAEDIATRIC SOFT TISSUE SARCOMA STUDY GROUP RMS 2005 STUDY

Isabelle S.A de Vries, MD[1]; Kieran McHugh, FRCR, FRCPI, FFRRCSI[2]; Gianni Bisogno, Prof. dr.[3]; Veronique Minard Colin, Prof. dr.[4]; Beatrice Coppadoro, BSc[3]; Ilaria Zanetti, n/a[3]; Andrea Ferrari, MD[5]; Carlo Morosi, MD[6]; Daniel Orbach, MD[7]; Salma Moalla, MD[8]; Myriam Ben-Arush, Prof. dr.[9]; Christine Devalck, MD, PhD[10]; Meriel E. Jenney, Prof. dr.[11]; Julia Chisholm, MD, PhD[12]; Gabriela Guillen Burrieza, MD, PhD[13]; Henry C. Mandeville, FRCR, MD(Res)[14]; Johannes H. M Merks, Prof. dr.[15]; Rick R. Van Rijn, Prof. MD. PhD.[16]; Roelof van Ewijk, MD[1], (1) Princess Maxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands, (2) Department of Radiology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, England, United Kingdom, (3) Department of Pediatric Haematology and Oncology, University Hospital of Padua, Padua, Veneto, Italy, (4) Gustave-Roussy, Université Paris-Saclay, Villejuif, Ile-de-France, France, (5) Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Lombardia, Italy, (6) Department of Radiology, Fondazione IRCSS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (7) SIREDO Oncology Center (Care, Innovation and Research for Children and AYA with Cancer), Institut Curie, and University PSL, Paris, Ile-de-France, France, (8) Department of Pediatric and Adolescent Oncology, Gustave-Roussy, Université Paris-Saclay, Villejuif, Ile-de-France, France, (9) Rambam Medical Center, Joan and Sanford Weill Pediatric Hematology Oncology and Bone Marrow Transplantation Division, Ruth Rappaport Children's Hospital, Haifa, Haifa, Hefa, Israel, (10) Department Hematology Oncology, HUDERF, ULB, HUB, Brussels, Belgium, Brussel, Brussels Hoofdstedelijk Gewest, Belgium, (11) Department of Paediatric Oncology, Children's Hospital for Wales, CARDIFF,

Wales, United Kingdom, (12) Children and Young Peoples Unit, Royal Marsden Hospital and Institute of Cancer Research, Sutton, England, United Kingdom, (13) Department of Pediatric Surgery, Hospital Infantil Universitari Vall d'Hebron, Barcelona, Catalonia, Spain, (14) Department of Radiotherapy, Royal Marsden Hospital and The Institute of Cancer Research, Sutton, Sutton, England, United Kingdom, (15) Princess Máxima Center for Pediatric Oncology and Division of Imaging and Oncology, University Medical Center Utrecht, University of Utrecht, Utrecht, Utrecht, Netherlands, (16) Department of Radiology and Nuclear Medicine, Amsterdam UMC – Emma Children's Hospital, University of Amsterdam, Amsterdam, Noord-Holland, Netherlands

Objective: In rhabdomyosarcoma (RMS), there is an ongoing search for early prognostic markers predicting outcome. First, to identify high-risk patients and therefore be able to intensify or de-intensify treatment at an early stage. Second, to early recognize effective new treatment strategies, and bring promising treatment to phase III while dropping inferior regimens. Currently, the only endpoint for trials available is survival, taking up to a decade to give results. In previous publications the prognostic value of early radiologic tumor size response has shown to be controversial.
We present the prognostic value of early tumor size response in a large cohort of non-metastatic RMS patients with residual disease after initial surgery or biopsy, Intergroup Rhabdomyosarcoma Studies (IRS) postsurgical Group III, treated according to state-of-the-art guidelines in the European paediatric Soft tissue sarcoma Study Group (EpSSG) RMS 2005 cohort.

Methods: For this post-hoc analysis, IRS Group III patients from the EpSSG RMS 2005 cohort with tumor volume measurements available at diagnosis and after three courses of induction chemotherapy were included. Measured tumor response was grouped according to the EpSSG RMS 2005 imaging protocol: complete response (CR), partial response (PR), minor partial response (mPR), stable disease (SD) and progressive disease (PD) (Figure 1). Overall survival (OS) was defined as time from tumor evaluation to death from any cause, and failure free survival (FFS) as time from tumor evaluation to disease progression, a second malignancy, or death. In FFS analyses, PD patients were excluded, as disease progression is within the definition of FFS. OS and FFS were estimated using the Kaplan-Meier method and comparisons between the response groups were made using the log-rank test. The prognostic impact was assessed using a univariate Cox proportional hazards model. Multivariate Cox regression analysis was used to evaluate the independent prognostic factors

Results: Of 1306 IRS Group III patients, 618 had all measurements available after three courses of induction chemotherapy and were included for analysis. 49 patients (8%) achieved CR, 373 patients (60%) PR, 140 patients (23%) mPR, 47 patients (8%) SD, and nine patients (2%) PD. The estimated five-year FFS and OS for all patients were respectively 67.3% (95% CI 63.4%-70.9%) and 80.7% (95% CI 77.4%-83.7%). No significant differences were observed for FFS or OS between the different response groups (Figure 2). Univariate Cox proportional hazards model showed no significant difference for FFS (p=0.140) and OS (p=0.252) between the different response groups. Hazard ratios (HR) for PR, mPR and SD were 0.97 (95% CI, 0.59-1.61), 1.05 (95%CI, 0.60-1.82) and 1.5 (95%CI, 0.79-2.79), respectively, for FFS and 1.17 (95% CI, 0.61-2.24), 1.35 (95% CI, 0.67-2.71) and 1.85 (95%CI, 0.85-4.04), respectively, for OS. In the multivariate cox regression analysis site (unfavorable; HR 1.54, 95% CI, 1.08-2.22), histology (unfavorable; HR 1.51, 95% CI, 1.13-2.01), and loco-regional lymph node involvement (N1; HR 1.48, 95%CI: 1.07-2.03) were identified as independent prognostic factor for FFS. Site (unfavorable; HR 2.92, 95% CI, 1.69-5.06 and loco regional lymph node involvement (N1; HR 2.39, 95%CI: 1.70-3.36) were identified as independent prognostic factor for OS as well. For both FFS and OS, tumor volume response was not identified as an independent prognostic

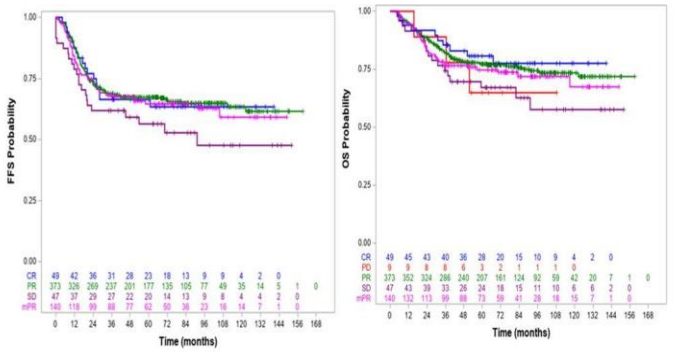
factor.

Conclusion: This study confirms that radiological tumor volume response after three courses of induction chemotherapy does not predict FFS or OS in IRS Group III patients treated according to the EpSSG RMS 2005 protocol and cannot be considered as an early surrogate marker in pediatric RMS. Therefore, there remains an urgent need to develop new imaging markers prognostic for outcome in RMS patients. This is the subject of imaging biomarker studies in the current Frontline and Relapse Rhabdomyosarcoma (FaR-RMS) study.

Figure 1. EpSSG RMS 2005 tumor volume response groups based on tumor volume reduction or increase.

Complete response	Complete disappearance of all visible disease
Partial response	Tumor volume reduction ≥ 66% but < 100%
Minor partial response	Tumor volume reduction ≥ 33% but < 66%
Stable disease	Tumor volume reduction <33% and increase <40%
Progressive disease	Any increase in volume ≥ 40%, or appearance of new lesions

Figure 2. Kaplan-Meier estimates of FFS and OS based on early tumor volume response of 618 IRS Group III patients included in the EpSSG RMS 2005 protocol. For FFS, PD patients were excluded. FFS, failure free survival; OS, overall survival; CR, complete response; PR, partial response; mPR, minor partial response; SD, stable disease; PD, progressive disease; IRS, Intergroup Rhabdomyosarcoma Studies; EpSSG, European *paediatric* Soft tissue sarcoma Study Group.



	N	Failed	5-yr FFS (95% CI)	p-value
CR	49	17	66.3 (51.0-77.8)	0.3392
PR	373	127	67.3 (62.2-71.9)	
mPR	140	50	64.7 (55.9-72.1)	
SD	47	22	56.3 (40.6-69.3)	

	N	Deaths	5-yr OS (95% CI)	p-value
CR	49	10	80.7 (66.1-89.5)	0.3935
PR	373	88	77.2 (72.5-81.3)	
mPR	140	37	74.8 (66.6-81.4)	
SD	47	17	67.0 (51.1-78.7)	
PD	9	3	64.8 (25.3-87.2)	

(P 210) **FINANCIAL TOXICITY ASSOCIATED WITH THE TREATMENT OF SARCOMAS: A SINGLE INSTITUTION RETROSPECTIVE-PROSPECTIVE STUDY**

Eric Shinohara, MD, MSCI; Elizabeth J. Davis, MD; Joshua M. Lawrenz, MD, MSCI; Michael J. Robinson, MD; Emma A. Schremp, MS; Tatsuki Koyama, PhD; Lili Sun, PhD, MS; Tuya Pal, MD; Ben H. Park, MD, PhD; Xiao-Ou Shu, MD, PhD; Debra L. Friedman, MD, MS; Scott Borinstein, MD, PhD, Vanderbilt University Medical Center, Nashville, TN

Objective: Financial toxicity has been linked to decreased quality of life and outcomes in cancer patients. Sarcoma patients can have long, complex treatment courses, which often involve some combination of surgery, chemotherapy, and radiation. Prior studies in countries with universal healthcare, such as Germany, have demonstrated higher levels of financial distress in patients with sarcoma, compared to an age and gender-matched general population. In the present study, sarcoma patients treated at Vanderbilt University Medical Center (VUMC) Sarcoma Treatment Center, which is amongst the largest sarcoma programs in the US, were evaluated for financial toxicity.

Methods: Participants were enrolled in the “Cohort to Augment the Understanding of Sarcoma Survivorship Across the Lifespan” (CAUSAL) study, a retrospective-prospective cohort study to understand sarcoma survivorship through the lifespan. Patient and tumor characteristics were abstracted from the electronic medical record. The Comprehensive Score for Financial Toxicity (COST) validated questionnaire was administered at enrollment to assess financial toxicity, where lower scores indicate higher financial toxicity.

Results: Of 736 participants enrolled in CAUSAL to date and treated between 2012 to present, 494 completed a COST questionnaire and are included in this analysis. Median COST score at enrollment was 28 (18-35). Higher financial toxicity was not associated with any patient

demographics including age, race, education level, and marital status. Higher financial toxicity was also not associated with various tumor or treatment-related factors, including number of treatments, receiving or having completed treatment, tumor size, or tumor location. Female participants demonstrated greater financial toxicity than male participants (p=0.045). The median COST score for participants described as “Out of Work” is 18. Compared with the participants employed for wages, financial toxicity was significantly higher in participants that reported they were temporarily out of work (p=0.044). The median COST score for participants with an income <$45,000 is 19. Participants with higher income were less likely to experience financial toxicity; all income levels above $45,000 demonstrated a p value < 0.01. Compared to participants with commercial insurance, financial toxicity score was significantly higher in participants with Medicaid (p=0.026), and significantly lower in participants insured with Medicare (p=0.022).

Conclusion: Higher levels of financial toxicity were associated with individuals who were out of work and had lower income. These findings may enable providers to better identify patients who are at risk for financial toxicity and connect them with resources. This may ultimately improve patients’ abilities to complete treatments as well as reduce stress. With ongoing accrual in CAUSAL, we will further evaluate predictors of financial toxicity in order to develop supportive interventions to improve patient outcomes.

(P 211) AN ANALYSIS OF SELF-REPORTED DEPRESSION IN SARCOMA PATIENTS

Michael J. Robinson, MD[1]; Sang Minh Nguyen, PhD[1]; Emma A. Schremp, MS[1]; Lauren King, MPH[2]; Jian Liang, BA[1]; Elizabeth J. Davis, MD[1]; Debra L. Friedman, MD, MS[1]; Ben H. Park, MD, PhD[1]; Xiao-Ou Shu, MD, PhD[1]; Tuya Pal, MD[1]; Scott Borinstein, MD, PhD[1], (1) Vanderbilt University Medical Center, Nashville, TN, (2) Vanderbilt University Medical Center, Greenbrier, TN

Objective: Sarcomas are a rare heterogeneous group of malignancies, with varied but often intensive treatment among different subtypes, including surgery, radiation, and systemic therapy. Such therapy is intended to improve survival but may also lead to adverse psychological and social outcomes. Scarce data exists correlating patient demographic and lifestyle characteristics to symptoms of depression in the contemporary treatment era. In a cohort of sarcoma patients treated with contemporary therapy, we evaluated prevalence rate and associated factors for self-reported symptoms of depression.

Methods: The “Cohort to Augment the Understanding of Sarcoma Survivorship Across the Lifespan” (CAUSAL) is designed to build a retrospective-prospective cohort of sarcoma patients to assess the impact of sarcoma, its treatment, and patient characteristics on health outcomes. Sarcoma patients treated at Vanderbilt University Medical Center from 2012-present are being identified and recruited to the study. Self-reported depression symptoms are assessed via the PROMIS-57, which encompasses seven domains across physical, mental, and social health in adults. We report on responses from CAUSAL participants to the PROMIS-57 depression survey, collected at the time of enrollment into CAUSAL. Scores from each completed survey are calculated into a raw score and translated into a T-score, allowing for standardization. The general population mean T-score is 50, with a standard deviation (SD) of 10. Higher scores are more associated with symptoms of depression. Patient demographic and lifestyle characteristics are collected via patient surveys. Clinical characteristics and sarcoma treatment

data were abstracted from the electronic medical record. Associations between participant characteristics and treatment with T-scores, per SD increase, were evaluated using multivariable linear regression analysis. Mean differences (β coefficients) and 95% confidence intervals (CIs) were derived from linear regression models.

Results: Our analysis includes 429 participants who completed the PROMIS-57 survey. There are more female (54.1%) than male participants. The median age is 55 (range 18-86). Among participants, 147 are receiving therapy, and 282 have completed treatment. Mean depression T-score was 48.1 (SD 9.7) in our study population; 48.3% of participants had a T-Score of 41-59 (within one SD of the mean of the general population). 39.4% had a T-score ≤40 (greater than one SD below the mean of the general population), while 12.3% scored ≥60 (greater than one SD above the mean of the general population). Multivariable linear regression showed that age, income and smoking were significantly associated with the depression score. Compared with participants >60 years, participants 18-39 and 40-59 years of age had significantly higher depression scores (β = 0.38, 95% CI: 0.12, 0.64, p = 0.005) and (β = 0.27, 95% CI: 0.07, 0.48, p = 0.010), respectively. Compared to participants with household incomes under $45,000, participants with higher household incomes had significantly lower depression scores: income $45,000-$70,000 (β = -0.34, 95% CI: -0.62, -0.06, p = 0.017), income $70,000-$120,000 (β = -0.50, 95% CI: -0.77, -0.23, p< 0.001) and income >$120,000 (β = -0.51, 95% CI: -0.81, -0.22, p< 0.001). Additionally, participants who identified as previous or current smokers had significantly higher depression (β = 0.21, 95% CI: 0.01, 0.40, p = 0.039), compared to those who did not. Participants' current treatment status and tumor characteristics, such as diagnosis and primary tumor site, were not independently associated with the depression score.

Conclusion: Our study is thus far the largest analysis evaluating depression symptoms among sarcoma patients. We found that younger sarcoma patients, previous or current smokers, and those with low income have a high prevalence of depression symptoms. These findings provide important information for developing personalized supportive care strategies.

(P 212) REPORT ON THE EWING SARCOMA PATIENTS ENROLLED IN ARM D OF THE ACSE-ESMART TRIAL: PHASE I/II STUDY OF THE PARP INHIBITOR OLAPARIB IN COMBINATION WITH IRINOTECAN IN CHILDREN WITH ADVANCED MALIGNANCIES

Susanne A. Gatz, MD/PhD[1]; Pablo Berlanga, MD/PhD[2]; Baptiste Archambaud[3]; Yassine Bouchoucha, MD[4]; Nicolas André, MD/PhD[5]; Nadege Corradini, MD[6]; Windy Rondof, Msc[2]; Jonathan Rubino, Msc[2]; Souad Nebchi, MChem[2]; ANTONIN MARCHAIS, PhD,HDR[7]; Estelle Thebaud, MD[8]; Alba Rubio San Simón, MD[9]; Natasha van Eijkelenburg, MD[10]; Lynley V. Marshall, MBBCh PhD[11]; Anne-Sophie Defachelles, MD[12]; Adela Canyete, MD[13]; Stephane Ducassou, MD/PhD[14]; Guy Makin, MBBCh PhD[15]; Michela Casanova, MD[16]; Emilie De Carli, MD[17]; Arnaud Petit, MD, PhD[18]; Gwenael Le Teuff, PhD[19]; Xavier Paoletti, PhD[20]; Peter Mortimer, PhD[21]; Gilles vassal, MD, PhD[22]; Birgit Geoerger, MD, PhD[23], (1) University of Birmingham, Birmingham, England, United Kingdom, (2) Gustave Roussy cancer Centre, Villejuif, Ile-de-France, France, (3) Gustave Roussy, VILLEJUIF, Ile-de-France, France, (4) Institute Curie, Paris, Centre, France, (5) La Timone Hospital, Marseilles, Provence-Alpes-Cote d'Azur, France, (6) Institut d'Hematologie et d'Oncologie Pédiatrique, Centre Léon Bérard, Lyon, Rhone-Alpes, France, (7) Gustave Roussy, villejuif, Ile-de-France, France, (8) Centre

Hospitalier Universitaire, Nantes, Pays de la Loire, France, (9) Hospital Nino Jesus, Madrid, Madrid, Spain, (10) Princess Máxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands, (11) The Royal Marsden Hospital, Sutton, England, United Kingdom, (12) Centre Oscar Lambret, Lille, Haute-Normandie, France, (13) Pediatric Hospital La Fe, Valencia, Catalonia, Spain, (14) Pediatric Hospital, Beaurdeaux, Aquitaine, France, (15) University of Manchester, Manchester, England, United Kingdom, (16) Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Lombardia, Italy, (17) Pediatric hospital of the University, Anger, Pays de la Loire, France, (18) APHP. Sorbonne Université, Hopital Armand Trouseau, PARIS, Ile-de-France, France, (19) Gustave Roussy Cancer campus, Villejuif, Ile-de-France, France, (20) Institute Curie, Paris, Ile-de-France, France, (21) AstraZeneca, Ely, England, United Kingdom, (22) Gustave Roussy, Villejuif, Ile-de-France, France, (23) Gustave Roussy Cancer Center, Université Paris-Saclay, INSERM U1015, Villejuif, Ile-de-France, France

Objective: Ewing sarcoma (ES) has been identified as a PARP inhibitor (PARPi) sensitive cancer in preclinical studies. The ES-specific fusion protein itself – best investigated for EWSR1-FLI1 – has been shown to be linked to this sensitivity by mimicking a BRCA deficient phenotype; however, the full mechanism of this sensitivity remains unknown. Despite this preclinical sensitivity, PARPi monotherapy in relapsed/refractory ES patients was unsuccessful and several combination therapies including PARPi - mainly in combination with chemotherapy - have been and are being investigated. AcSé-ESMART is a proof-of-concept, phase I/II, platform trial, designed to explore targeted agents in a molecularly enriched relapsed/refractory pediatric population. Arm D was evaluating the PARPi olaparib in combination with the topoisomerase I inhibitor irinotecan. In contrast to other PARPi/chemotherapy combination studies, we opted for a prolonged course of PARPi and low dose irinotecan as sensitizer. The Phase I part established the recommended Phase II dose (RP2D) (Gatz ASCO 2019). The Phase II part of the trial assessed the activity in two separate expansion cohorts (cohort 1: homologous recombination repair defect (HRD) and cohort 2: ES). We here report the results of all ES patients enrolled in this study.

Methods: Comprehensive molecular profiling (WES -/+ RNA sequencing) (ideally from the relapsed tumour) was required as part of the inclusion criteria for all patients. Patients with ES were eligible based on the presence of an ES-specific fusion. Dose escalation followed a continuous reassessment method (CRM) with a dose limiting toxicity (DLT) threshold of ≤0.25. In the escalating dose levels Olaparib was administered orally twice daily at 90-130 mg/m2 on Days 1 to 10 and irinotecan intravenously at 10-30 mg/m2 on Days 4 to 8 of a 21-day cycle. Starting dose level (DL1) was Olaparib 90 mg/m2 on Days 1 to 10 and irinotecan 10 mg/m2 on Days 4 to 8 of a 21-day cycle. Adverse events (AEs) were reported based on National Cancer Institute Common Toxicity Criteria (NCI-CTCAE) version 4.03. Activity assessment in the two expansion cohorts followed a Minimax Simon 2-stage design. Each cohort was to progress to the second stage (additional 9 patients) if 2 or more confirmed responses were observed in the first 16 patients. Patients treated in the Phase I part at the RP2D were counting towards the respective expansion cohorts.

Results: From October 2016 till July 2022 seventy patients (median age: 14 years, range 5-23) in total were included in the study, 36 of them were ES patients; there were four screen failures – all were ES patients: three were not treated due to rapid progressive disease, in one case the patient declined treatment. Four dose levels were explored in the phase I part to identify the RP2D (DL1, DL2, DL3 and DL2a). The RP2D was DL2 (Olaparib 90 mg/m2 and irinotecan 20 mg/m2). Of the 32 treated ES patients, eight were enrolled in the Phase I part (DL1 n=3; DL2 n=2; DL3 n=2; DL2a n=1) and 24 in the Phase 2 part. Six patients had DLTs, all at DL2 (Phase I part n=1; Phase 2 part, n=5) for an overall DLT rate at DL2 in the ES cohort alone of 0.23. Main AEs including the DLTs were hematological and gastroinstestinal and not different from

the non-ES patients. All patients had an ES fusion (ESWR1::FLI1 n=25; EWSR1::ERG n=4; EWSR1 translocation by FISH n=2; data missing n=1). Median number of treatment cycles were 2, range 2;40+. Three patients had objective responses, two of them confirmed: one patient - treated on DL1 - had an unconfirmed partial response (PR) and received 6 cycles; on DL2, one patient had a complete response (10 cycles) and one a PR; this patient is ongoing in cycle 40. Nine patients had stable disease (SD) as best response, three of them prolonged with 6, 10, 16 cycles, respectively (all in DL2). Retrospective correlative analysis of the molecular profiling data and additional tumor tissue expression analysis are ongoing to identify potential constellations linked with these exceptional responses and rapid progression, respectively.

Conclusion: The combination of Olaparib and irinotecan in this protracted schedule is overall well tolerated. Encouraging clinical benefit was observed in a subset of ES patients with one patient remaining on treatment 2.5 years after enrolment. Current molecular hypothesis is insufficient for patient selection and better biomarkers are needed.

(P 213) EXPERIENCE WITH THE USE OF TRABECTEDIN IN ADVANCED SOFT TISSUE SARCOMA IN IRELAND

Laurann Rabbitt, MB BCh BAO (hons)[1]; Catherine S Weadick, n/a[2]; Catherine Molloy, n/a[3]; Deirdre O'Mahony, MB Bch BAO, MSc, FRCPI[4]; Caitriona Goggin, MB BCh BAO, MSc, MRCPI[5]; Richard Bambury, n/a[2]; Sinead Noonan, n/a[2]; Paula Calvert, n/a[6]; Hazel L. Murray[7]; Tadhg Reddan[8]; John Crown, n/a[9]; David Fennelly, n/a[9]; Mark Doherty, MB BCh BAO MSc MRCPI[10], (1) SVUH, Dublin 8, Dublin, Ireland, (2) CUH, Cork, Cork, Ireland, (3) SVUH, Dublin, Dublin, Ireland, (4) Bon Secours, Cork, Cork, Cork, Ireland, (5) University Hospital Waterford, Waterford, Waterford, Ireland, (6) UHW, Waterford, Waterford, Ireland, (7) St Vincents University Hospital, Elm Park, Dublin 4, Ireland, Dublin, Dublin, Ireland, (8) SVUH, Dublin 4, Dublin, Ireland, (9) SVHG, Dublin, Dublin, Ireland, (10) St Vincents University Hospital, Dublin 4, Dublin, Ireland

Objective: Soft tissue sarcoma (STS) is comprised of 80 pathologic subtypes based on a combination of distinctive histological features. Adult-type soft tissue and visceral sarcomas are rare, with an estimated incidence of 4-5/100,000 per year in Europe. Trabectedin is licensed for advanced soft-tissue sarcoma in Europe, after failure of anthracyclines and ifosfamide, or for patients who are unable to receive these agents. Our aim of this project is to retrospectively analyse the use of trabectedin in an Irish cohort and assess its efficacy and tolerability.

Methods: All adult patients who received at least one cycle of trabectedin in the palliative setting for soft tissue sarcoma between June 2014 and June 2023 were retrospectively analysed across five Irish cancer centres. Data regarding clinicopathological characteristics, toxicity and outcomes were collected. Overall survival (OS) and progression-free survival (PFS) was recorded and assessed using Kaplan Meier method for time to event analysis.

Results: One hundred and sixteen patients were included in the overall analysis, with a median age of 57 (range, 23-82 years). The most common subtypes were leiomyosarcoma (n=46), liposarcoma (n=16), synovial sarcoma (n=12), and pleomorphic sarcoma (n=8), with a further 24 subtypes with less than five patients each. The median number of previous palliative chemotherapy regimes was 1 (range, 0-5), with 53% of patients (n=62) having received an

anthracycline in the palliative setting. Patients received a median number of 3 cycles of trabectedin (range, 1-40). Median PFS and OS of the entire cohort was 2.5 months (range 0-30 months) and 7 months (range 0-67 months), respectively. This varied considerably by subtype. Patients with leiomyosarcoma or liposarcoma (abbreviated as L-type sarcoma) had a progression free survival of 3 months (range 0-67 months), compared to 2 months (range, 0-21 months) for all other subtypes ($p< 0.05$). For patients with toxicity data collected (n=67), 10% stopped treatment due to toxicity, 31% had an unscheduled admission and 14.9% required a dose reduction.
There were 26 patients total with a progression free survival of over six months. They had received a median of 1 line of previous systemic treatment in the palliative setting (range 0-2). They had diagnoses of soft tissue leiomyosarcoma (n=9), myxoid liposarcoma (n=8), poorly differentiated sarcoma (n=3), uterine leiomyosarcoma (n=2), and one patient each with dedifferentiated liposarcoma, solitary fibrous tumour, synovial sarcoma and sclerosing epithelioid fibrosarcoma. They received a median of 12 cycles of trabectedin with a median PFS of 13 months and OS of 38 months. Of those who had data collected regarding toxicity (n=14), five (35.7%) required a dose reduction.

Conclusion: This retrospective real-world study underlines trabectedin's role as a well-tolerated second line treatment in advanced sarcoma. Further studies are required to identify the patients who may benefit most from trabectedin.

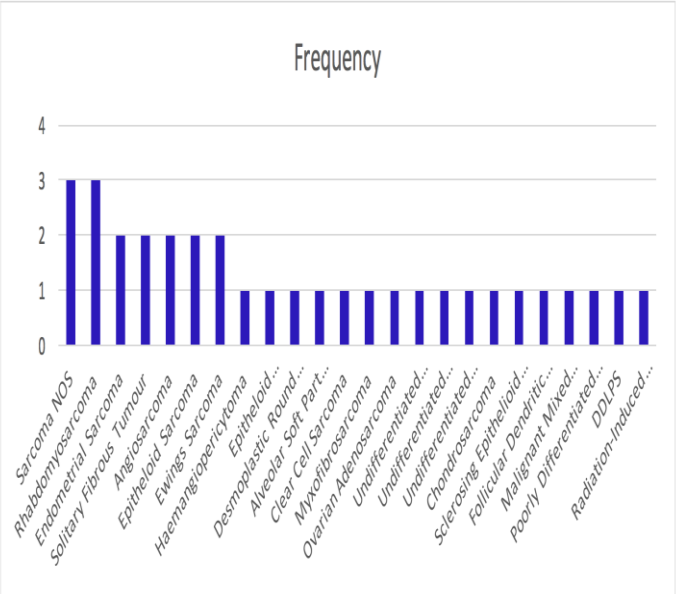


Figure 1. Frequency of histopathological subtypes

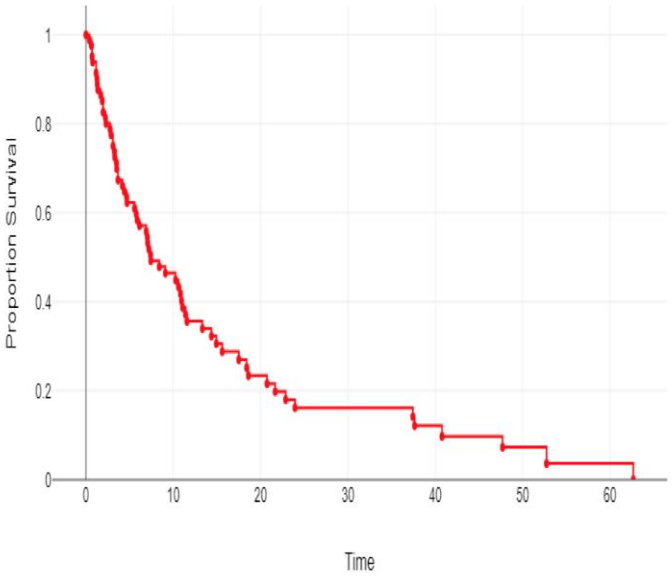


Figure 2. Overall survival (months)

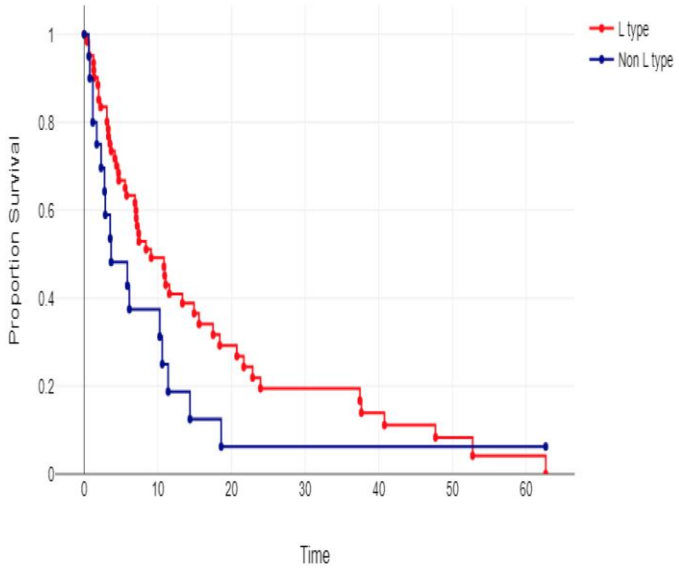


Figure 3. Overall survival in L-type versus all other soft tissue sarcoma subgroups (months)

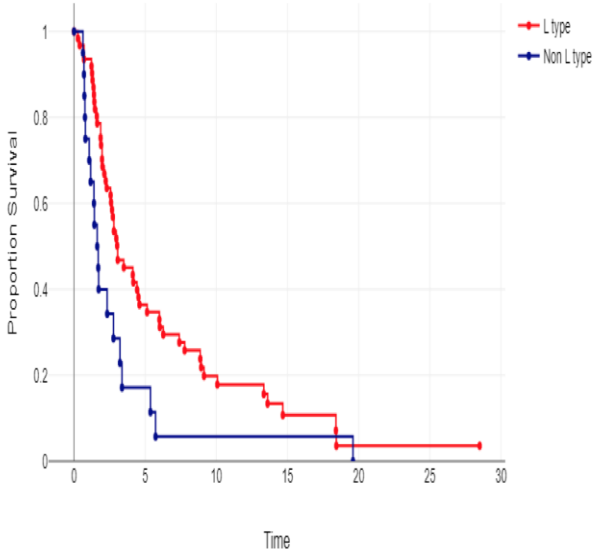


Figure 4. Progression free survival in L-type versus all other soft tissue sarcoma subgroups (months)

(P 214) SIGNIFICANT TREATMENT RESPONSE IN PATIENTS WITH PRIMARY RESECTABLE EXTREMITY MYXOID LIPOSARCOMA RECEIVING NEOADJUVANT TRABECTEDIN AND RADIATION

Corey Hounschell, DO[1]; Dylan Vance, BS[2]; Kyle Sweeney, MD[2]; Elizabeth Friedman, MD[3]; Howard G. Rosenthal, MD[4]; Ronny Rotondo, MD[5]; Benjamin C. Powers, MD[6]; Luke Selby, MD MS FACS[3], (1) University of Kansas, Department of Surgery, Overland Park, KS, (2) University of Kansas Medical Center, Kansas City, KS, (3) University of Kansas, Kansas City, KS, (4) University of Kansas Health System, Overland Park, KS, (5) Kansas City Medical Center, Kansas City, KS, (6) The University of Kansas Health System, Leawood, KS

Objective: Improved understanding of histology-specific sarcoma behavior and treatment has lead to improved disease-specific treatment. Historically all patients with extremity sarcoma received histology-agnostic neoadjuvant radiation or cytotoxic chemotherapy. More recently, Sanfilippo et al demonstrated that patients with myxoid liposarcoma who received neoadjuvant trabectedin combined with neoadjuvant radiation experienced significant rates of pathologic complete response at surgical resection. We now treat patient with extremity myxoid liposarcoma who are eligible for limb salvage with a similar protocol of neoadjuvant trabectedin combined with neoadjuant radiation. To date two patients have undergone this treatment regimen, clinical and pathological characteristics with short-term follow up of these patients are summarized.

Methods: Two patients with a primary resectable extremity myxoid liposarcoma received neoadjuvant trabectedin (initially: 1.5mg/m2 for six cycles) combined with neoadjuvant radiation (50 cGy in 25 fractions) during cycles five and six. Following recovery from neoadjuvant therapy patients were taken for surgical resection. Pathologic response and tumor cellularity of the

initial biopsy specimen and of the resected specimen were defined by board certified sarcoma pathologists. Treatment and operative characteristics were recorded.

Results: The use of neoadjuvant trabectedin in two patients with primary resectable extremity myxoid liposarcoma is associated with significant treatment response (< 5% and < 1% viable tumor) and substantial decrease in tumor size from preoperative imaging to final surgical specimen (10.5cm to 7.2cm and 9.7cm to 4.2cm). One patient required trabectedin dose reduction to 1.2mg/m2 for cycles 4-6 due to elevated liver enzymes. Neither patient has experienced short term local or distant recurrence. Both patients were able to work while receiving neoadjuvant therapy.

Conclusion: The use of neoadjuvant trabectedin and neoadjuvant radiation in patients with primary resectable extremity myxoid liposarcoma is associated with significant treatment effect with no impact on operative outcomes and no recurrence during short-term follow up. Longer follow up with more patients and comparison to historical control patients receiving neoadjuvant radiation alone is needed to understand the impact on long term recurrence and survival, but given the established association between pathologic complete response and overall survival this regimen is our current standard for patients who present with resectable myxoid liposarcomas with round cell features.

Table 1: Clinical and Pathological Characteristics of patients receiving neoadjuvant trabectedin combined with radiation.

	Patient #1	Patient #2
Anatomic Location	Thigh	Thigh
Size at diagnosis (cm)	10.5	9.7
Size at surgical resection (cm)	7.2	4.2
% Cellularity	<1% viable tumor	<5% viable tumor
% Round Cell (pre treatment)	>20%	>30%
% Round Cell (post treatment)	<1%	<1%
Post-Operative wound complications	None	None
Follow Up	98 days	82 days
Clinical Status	NED	NED

Figure 1: Patient #1 pre and post-treatment histology

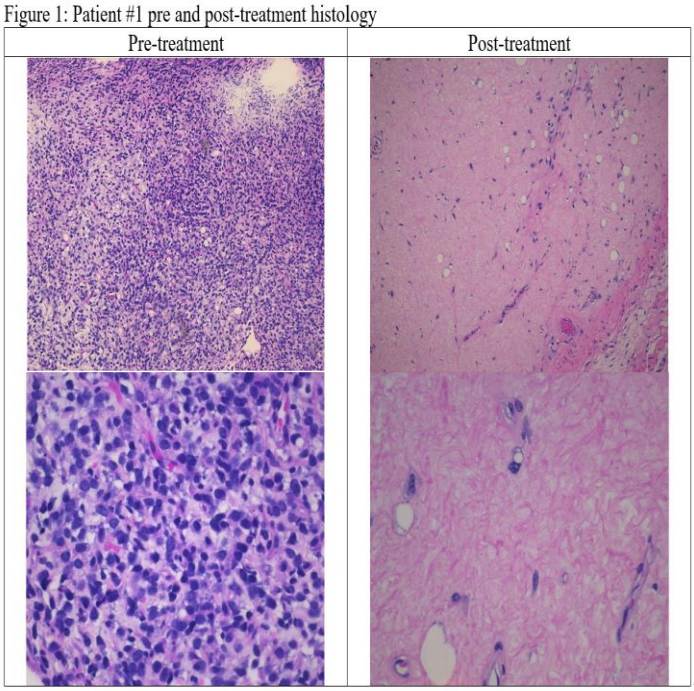
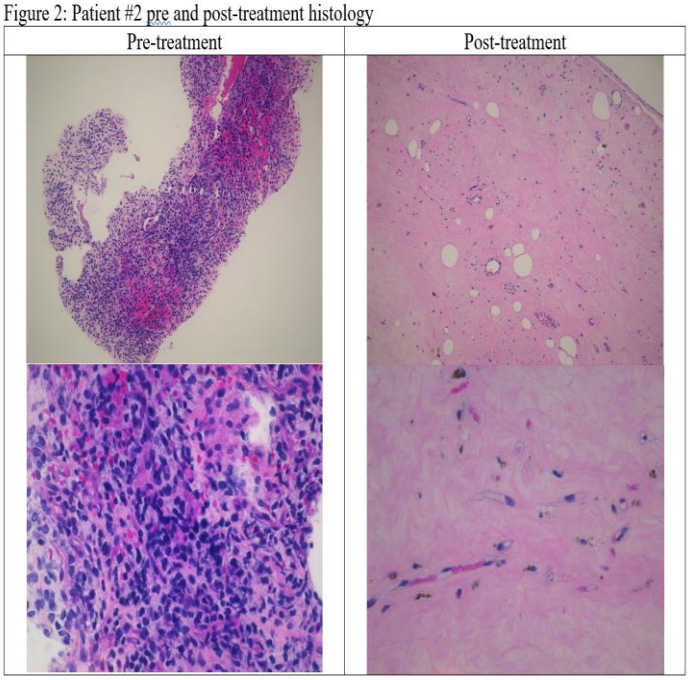


Figure 2: Patient #2 pre and post-treatment histology



(P 215) IMPACT OF FRONTLINE SYSTEMIC TREATMENT CHOICE ON CLINICAL OUTCOME IN ADVANCED LEIOMYOSARCOMA: A MULTI-CENTRE RETROSPECTIVE CANSARCC STUDY

Erica C. Koch Hein, MD[1]; Abha Gupta, MD MSc[2]; Hagit Peretz Soroka, Ph.D.[3]; Caroline L. Holloway, MD[4]; Brookelyn Biffart, Medical student[5]; Carolyn Nessim, MD[6]; Tristan Wild, BMSc[7]; Katherine Lajkosz, MSc[8]; Geoffrey Watson, MD[9]; Albiruni Ryan Abdul Razak, MB BCh, BM BCh[2]; Abdulazeez Salawu, MBBS MSc MRCP[UK] PhD[10], (1) University of Toronto, Toronto, ON, Canada, (2) Princess Margaret Cancer Center, Toronto, ON, Canada, (3) Princess Margaret Cancer Centre / The Hospital For Sick Children, Toronto, ON, Canada, (4) BC Cancer, Victoria, BC, Canada, (5) University of British Columbia, Victoria, BC, Canada, (6) Department of Surgery, University of Ottawa, Ottawa, ON, Canada, (7) University of Ottawa, Ottawa, ON, Canada, (8) University Health Network, Toronto, ON, Canada, (9) Mount Sinai Hospital / University of Toronto, Toronto, ON, Canada, (10) Mount Sinai Hospital and Princess Margaret Cancer Centre/University of Toronto, Toronto, ON, Canada

Objective: Leiomyosarcoma (LMS) is an aggressive soft tissue sarcoma (STS) subtype with the uterus as the most common anatomical site of origin. Commonly used frontline chemotherapy regimens for advanced STS are doxorubicin- or gemcitabine-based, with doxorubicin monotherapy widely regarded as standard. Combination chemotherapy with gemcitabine and docetaxel remains a popular choice over doxorubicin despite data from the randomized phase 3 GeDDIS trial that demonstrated no difference in survival between the 2 regimens. In this multi-centre, retrospective study, we aimed to evaluate whether the choice of frontline treatment influences clinical outcome in advanced LMS patients in a real-world setting.

Methods: Advanced LMS patients (uterine and non-uterine) who received frontline systemic treatment with doxorubicin- or gemcitabine-based chemotherapy at 4 Canadian sarcoma centres between 2010 and 2022 were included. Data were extracted from the curated and ethics board-approved Canadian Sarcoma Research and Clinical Collaboration (CanSaRCC) database. The primary and secondary endpoints were overall survival (OS) by choice of frontline treatment regimen and by the sequence of systemic treatments in the front and second lines, respectively. Survival analyses were performed using Kaplan-Meier method and differences in time-to-event endpoints between patient groups were assessed using the log-rank test, with $p < 0.05$ considered significant.

Results: A total of 217 patients (median age 58 years, range 28 – 92 years) were included (summarized on Table 1), among whom 102 (47%) had primary uterine LMS (uLMS). Median follow up was 15.4 months (range 0.4 – 84.1 months). Frontline systemic treatment was doxorubicin monotherapy in 61 patients (28%) and gemcitabine + docetaxel in 83 patients (38%) with median OS of 19.2 and 14.8 months, respectively ($p = 0.21$). Median OS was 17.8 months in patients who received frontline doxorubicin-based (n = 103) versus 16.1 months with gemcitabine-based (n=114) treatment ($p = 0.17$). In the uLMS subgroup, median OS was 15.2 versus 12.3 months with frontline doxorubicin-based (n = 43) and gemcitabine-based (n = 59) treatments, respectively ($p = 0.23$), while non-uLMS patients treated with frontline doxorubicin-based (n = 55) and gemcitabine-based (n = 55) treatments had median OS of 25.2 and 21.5 months, respectively ($p = 0.72$). Regarding sequence of treatments, patients who received frontline doxorubicin monotherapy followed by second-line gemcitabine + docetaxel (n = 18) had median OS of 22.0 versus 18.2 months among those patients (n = 18) who were treated with the reverse sequence ($p = 0.06$).

Conclusion: OS was numerically longer with frontline doxorubicin compared with gemcitabine + docetaxel in all LMS, but the difference was not statistically significant, in keeping with clinical trial data. Frontline doxorubicin followed by gemcitabine + docetaxel did not lead to longer survival than the reverse sequence. There was no difference in overall survival between patients who received frontline doxorubicin-based treatment compared to those who received gemcitabine-based treatment, regardless of primary LMS location. Differences in toxicity, quality of life, and cost of treatment delivery between regimens should therefore be considered in the choice of frontline treatment for advanced LMS patients.

Table 1: Baseline characteristics of included patients.

Characteristic - n (%)	n = 217
Sex, n (%)	
Female	168 (77.4)
Male	49 (22.6)
Age	
Median (min, max)	58 (28, 92)
Sarcoma origin	
LMS Uterus	102 (47.0)
LMS Non-Uterus	115 (53.0)
First line treatment	
Gemcitabine-based	
Gemcitabine + Docetaxel	83 (38.2)
Gemcitabine monotherapy	31 (14.3)
Doxorubicin-based	
Doxorubicin monotherapy	61 (28.1)
Doxorubicin + Olaratumab	17 (7.8)
Doxorubicin + Dacarbazine	1 (0.5)
Doxorubicin + Ifosfamide	12 (5.5)
Clinical trial including Doxorubicin	8 (3.7)
Liposomal Doxorubicin	2 (0.9)
Liposomal Doxorubicin + Olaratumab	2 (0.9)

(P 216) BRAIN METASTASES IN SARCOMA: A (NOT SO) RARE EVENT. THE EXPERIENCE OF A HIGH VOLUME CENTER

Fabio Murtas, MD[1]; Benedetta Chiusole, MD[2]; Ilaria Tortorelli, MD[2]; Antonella Galiano, MD[2]; Maria Samaritana S. Buzzaccarini, n/a[3]; Giovanni Scarzello, n/a[3]; Francesco Volpin, n/a[4]; Antonio Di Maggio, n/a[5]; Salvatore Vizzaccaro[2]; Chiara De Toni, n/a[2]; Antonella Brunello, MD, PhD[6], (1) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Decimoputzu, Sardegna, Italy, (2) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Veneto, Italy, (3) Radiation Therapy Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padova, Veneto, Italy, (4) Neurosurgery Unit, Azienda Ospedale-Università di Padova, 35128 Padova, Italy., Padova, Veneto, Italy, (5) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padova, Veneto, Italy, (6) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy, Padua, Veneto, Italy

Objective: Occurrence of brain metastases (BM) in patients with soft tissue (STS) and bone sarcoma (BS) is not common. Global incidence is estimated to be around 1-3% but data may differ according to histology, with alveolar soft tissue sarcoma, Ewing sarcoma, angiosarcoma and leiomyosarcoma showing the highest tendency to central nervous system spreading (up to 36%, 32%, 25% and 19% respectively). Brain involvement in sarcoma is considered an unfavorable event for prognosis, but highly variable survival rates are reported in literature.

Methods: This is a single Center retrospective study. We retrieved data of pts with STS and BS treated between 2010 and 2022 at Veneto Institute of Oncology from a retrospectively maintained database. The following data were collected: age at diagnosis, gender, primary tumor characteristics, date and location of first metastasis, time to BM, BM characteristics (number, location, edema), associated symptoms, management of BM, and survival. Pts lost at follow-up were censored at the last date known to be alive. Overall Survival (OS) was calculated with Kaplan-Meier method.

Results: Out of a total of 1870 pts, of whom 801 with advanced disease, 24 developed BM (1.3%; 3% considering only pts with advanced/metastatic disease). Pts' characteristics are summarized in Table 1. Most frequent histotypes among pts with BM were Ewing sarcoma (16.7%), leiomyosarcoma (12.5%), and undifferentiated pleomorphic sarcoma (UPS) (12.5%). BM were detected in 4 pts (16.7%) with Ewing sarcoma, 2 pts (6.1%) with synovial sarcoma), 2 pts (4.8%) with angiosarcoma , 2 pts (4.5%) with osteosarcoma (2pts) and 3 pts (4.0%) with UPS. Median time to onset of BM was 11 months from diagnosis and 4.6 months from the development of other metastatic sites. Most BM were diagnosed after the occurrence of symptoms (16pts, 66.6%); most associated symptoms were due to specific localisations rather than intracranial hypertension: a decline in vision and hyposthenia of the extremities (20.8% each), seizures and headache (16.7% each). In fact, only in 8 pts (33.3%) BM were associated with parenchymal edema. Sixteen pts (66.7%) developed metastasis only in the brain, 3 pts (12.5%) in the brain and cerebellum, 5 pts (20.83%) in the meninges. Half of the pts presented one single metastasis, 29.2% of pts had more than 5 sites; meningeal metastases were always widespread. [Table 2] BM were the only metastatic site for 5 pts (20.1%). Eighteen pts (75%) received at least one treatment for BM: 6 pts received only a local treatment, 6 pts received only systemic therapy and 6 pts both local and systemic therapy. Exclusive radiotherapy was performed in 5 pts, surgical resection in 4 pts, and a combination of radiotherapy and surgery in other 3 pts. Median OS from the date of brain metastasis was 2.4 months [95%CI: 1.88-NA], median OS from initial diagnosis was 17.4 months [95%CI: 12.50-NA]. At 12 months, 15 out of 24 patients have died: the probability of survival was 25.9% [95%CI: 11.98%-55.94%]. [Figure 1] No difference in OS according to sex, age and presence/absence of symptoms was found.

Conclusion: In our large series of patients with STS and BS, global incidence of BM was low, with some histological subtypes confirmed to be associated with higher risk, particularly Ewing sarcoma, synovial sarcoma, angiosarcoma, osteosarcoma and UPS. Most BM were single and amenable to local approach (surgery, radiotherapy or a combination of both). Despite best local and systemic treatment, BM still impact negatively on prognosis, with median OS from BM diagnosis being less than 3 months.

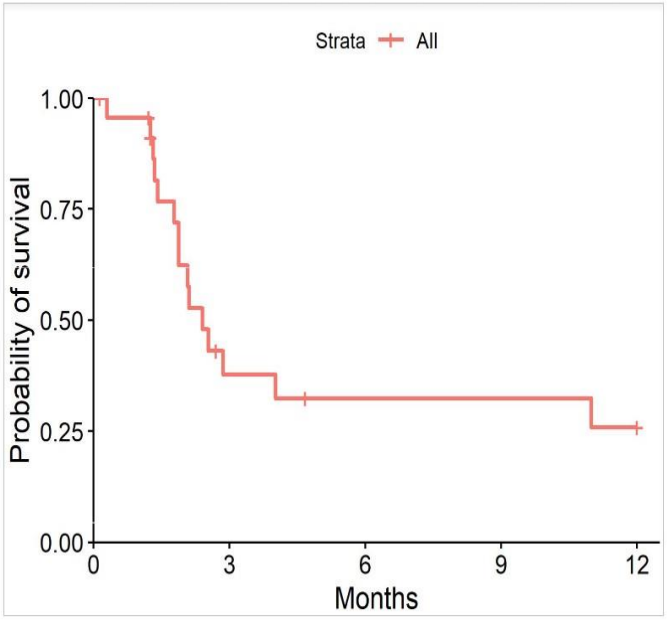
Table 1. Patients' characteristics

	n=24	%
Male	15	62.5
Female	9	37.5
Median age (diagnosis) **Median age BM**	58 (15-85) 59 (25-86)	
HYSTOTYPES		
Ewing Sarcoma	4	16.7
Leiomyosarcoma	3	12.5
UPS	3	12.5
Synovial sarcoma	2	8.3
Angiosarcoma	2	8.3
Osteosarcoma	2	8.3
Chondrosarcoma	2	8.3
Liposarcoma	1	4.2
MPNST	1	4.2
Other	4	16.7
PRIMARY TUMOR LOCATION		
Extremities	12	50
Head and neck	5	20.8
Lung	3	12.5
Other	4	16.7
PRIMARY		
Bone	5	20.8
Soft tissue	19	79.2

Table 2. BM characteristics

NUMBER OF BM		
Single	12	50
Multiple	12	50
LOCATION OF BM		
Brain	16	66.7
Meninges	5	20.8
Brain+Cerebellum	3	12.5
NUMBER OF BM		
1	12	50
≤5	5	20.8
>5	7	29.2
DIAGNOSIS OF BM		
Incidental	6	25
Symptoms	16	66.7
NA	2	8.3
SYMPTOMS		
Decline in vision	5	20.8
Hyposthenia of the extremities	5	20.8
Seizures	4	16.7
Headache	4	16.7
Unstable march	2	8.3
Confusion	2	8.3
Vomit	2	8.3
Dizziness	1	4.2

Figure 1. Probability of survival



(P 217) CONTEMPORARY TREATMENT PATTERNS AND OUTCOMES IN THE MANAGEMENT OF ANGIOSARCOMA IN IRELAND

Hazel L. Murray[1]; Mark Doherty, MB BCh BAO MSc MRCPI[2]; Una Hayden, RN HDip MSc[3]; Aine Sheridan, RGN, PG DIp[4]; Paul Ridgway, n/a[3]; Gary O'Toole, n/a[4]; alan Molloy, n/a[4]; David Healy, n/a[4]; Andrea Marnell, n/a[5]; Charles Gillham, MRCP FRCR MD[6]; Naomi Lavan, n/a[6]; Eric heffernan, n/a[4]; Aurelie Fabre, n/a[4]; Tom Crotty, n/a[4]; Amy Gillis, MD MCh[3], (1) St Vincents University Hospital, Elm Park, Dublin 4, Ireland, Dublin, Dublin, Ireland, (2) St Vincents University Hospital, Dublin 4, Dublin, Ireland, (3) Tallaght University Hospital, Dublin, Dublin, Ireland, (4) St Vincents University Hospital, Dublin, Dublin, Ireland, (5) Cappagh National Orthopaedic Hospital, Dublin, Dublin, Ireland, (6) St Luke's Radiation Oncology Network, Dublin, Dublin, Ireland

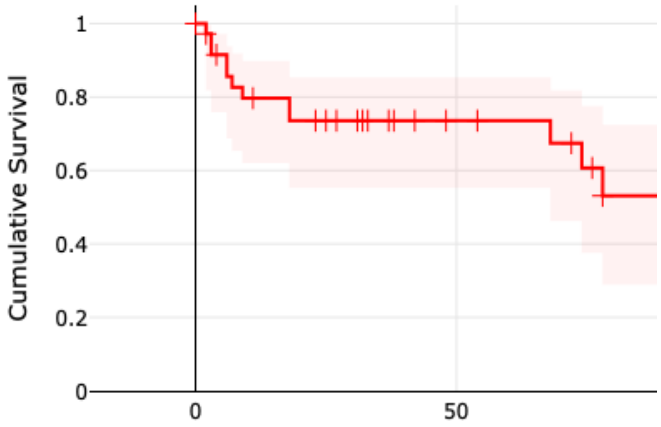
Objective: Angiosarcomas are known to be highly aggressive tumours often with poor outcomes. Surgery is the only potential curative therapy, but radiotherapy (RT), chemotherapy and targeted therapies may also of benefit in the adjuvant and metastatic setting. Overall, there is often a nihilistic view to disease modifying therapy. With an all-island national MDT view of the disease, we present a contemporary series of angiosarcoma.

Methods: All patients with a diagnosis of angiosarcoma who were discussed at the adult / AYA sarcoma MDM between 2017 and 2023 were included in the study. Data were collected on patient demographics, disease characteristics, treatment administered and outcome. Overall survival was calculated using the Kaplan-Meier method.

Results: A total of 39 patients were identified: 25 female and 16 male with a median age of 56 (range 17-89). 53% (n=21) presented with localised disease and 18% (n=7) of patients had

metastatic disease on presentation. 28% (n=11) of patients had a history of a previous cancer to the region and were identified as having a radiation induced angiosarcoma (RIA). Primary tumour site was breast in 28% (n=11), scalp in 13% (n=5), bone in 10% (n=4), head/neck in 10% (n=4) chest/abdominal wall in 15% (n=6), pericardial in 5% (n=2) 7 had other primary sites. Median tumour size was 50 mm (range 2-180mm). Tumour grade was available in 27 patients; 62% (n=24) had high grade tumours, 5% (n=2) low grade and 1 patient had intermediate grade. One patient had a de novo germline p53 mutation and another had Stewart Treves Syndrome; no other patients had familial genetic syndromes. Among 11 patients with RIA, 10 had prior breast cancer, and 1 had prior cervical cancer; this patient developed an abdominal wall tumour.
Within the RIA group 81% (n=9) had completion mastectomy, 1 had wide excision and 1 patient died less than 4 weeks from diagnosis with no further treatment. 36% (n=4) proceeded to adjuvant systemic therapy followed by adjuvant RT and 1 patient had adjuvant chemotherapy alone. Systemic treatment included Dox/Ifos in 3 patients, paclitaxel in 2 and propranolol in 1. 45% (n=5) relapsed more than 10 years from original diagnosis. 72% (n=8) are alive and free from disease at time of reporting. Among the other patients, 82% (n=23) had upfront surgery. 21% (n=6) received adjuvant RT, 14% (n=4) adjuvant chemotherapy and 7% (n=2) declined adjuvant therapy. 35% (n=10) are known to be alive at time of reporting. 32% (n=9) RIP. 21% (n=6) in the recurrent/metastatic setting received at least 1 or more lines of systemic therapy with standard regimens.

Conclusion: The management of patients with angiosarcoma is complex and should be managed through an expert centre. Irish patients treated with surgery and adjuvant therapy had outcomes comparable to international standards in this aggressive cancer. These data would suggest radical surgery and adjuvant therapy should be offered to all patients with localised radiation-induced angiosarcoma. Collaboration with regional centres is vital to the best management of patients with this rare disease.



(P 218) NEOADJUVANT CHEMOTHERPAY AND RADIATION DECREASES TUMOR SIZE, VIABILITY, AND RECURRENCE IN MULTIPLE LIPOSARCOMA SUBSETS

Dixon Dorand, Jr., MD, PhD[1]; Audrey Nicholls, BS[2]; Alden J. Dunham, BS[3]; Joshua M. Lawrenz, MD, MSCI[2]; Leo Luo, MD[4]; Eric Shinohara, MD, MSCI[2]; Carlos N. Prieto Granada, MD[2]; Ben H. Park, MD, PhD[2]; Elizabeth J. Davis, MD[2], (1) Tennessee Oncology, Nashville, TN, (2) Vanderbilt University Medical Center, Nashville, TN, (3) Vanderbilt University Medical Center, Tampa, FL, (4) Vanderbilt University Medical Center, Nsahville, TN

Objective: Soft tissue sarcomas are a diverse group of cancers derived from mesenchymal cells that comprise less than 1% of cancers. Liposarcomas (LPS) are one of the most common types of soft tissue sarcomas and arise from lipocytes of soft tissues with different subgroups: well-differentiated (WDLPS), dedifferentiated (DDLPS), myxoid/round cell (MRCLPS), and pleomorphic (pLPS). Surgical resection is the mainstay of treatment for LPS; however, the roles of neoadjuvant radiation (na-RT) and chemotherapy (na-CT) are less clearly defined, particularly in intermediate/high grade LPS. In this study, we sought to understand how neoadjuvant treatments (NAT) administered affected outcomes of patients with localized non-well-differentiated LPS.

Methods: Using Vanderbilt University Medical Center's Research Derivative, we identified patients with non-WDLPS who underwent resection of their primary tumor and were considered for neoadjuvant therapy from 01/2010 to 05/2022. Tumor characteristics and patient outcomes including margin status (PM), local recurrence (LR), and distant recurrence (DR) were assessed.

Results: 79 evaluable patients with DDLPS (43), MRCLPS (25), and pLPS (11) who underwent resection were identified (Table 1). Mean age was 57 years, 92.4% were white, 60% were male. 49% of patients had extremity tumors, 48% were intrabdominal or intrapelvic (IA/IP), 2% were in the chest or abdominal wall (CW/AW), and 1% were in the head or neck (H/N). 40 (51%) patients did not receive neoadjuvant therapy (no NAT), 27 (34%) received na-RT, and 6 (7.5%) patients received na-CT, and 6 (7.5%) received both na-RT and na-CT. 34 (43%) of patients had PM, 34 (43%) had LR, and 29 (36%) had DR. 24 (63%) of IA/IP tumors, 9 (23%) of UE/LE tumors, and 1 (100%) of H/N tumors had PM at resection. 20 of 34 (59%) of patients with PM had LR and 16 (47%) had DR.

27 patients (13 DDLPS, 9 MRC, 5 pLPS) received na-RT alone. Median tumor size was 15.5 cm pre-NAT and 15.8 cm post-NAT. Median MRCLPS tumor size was 14.7 cm pre-NAT and 13 post-NAT. Median viable tumor was 100% pre-NAT and 45% post-NAT: DDLPS 90% and 70% viable pre- and post-RT; MRC 100% and 10%; pleomorphic 100% and 30%. 33.3% had PM, 37% had LR, and 40.7% had DR.

6 patients (2 DDLPS, 2 MRCLPS, 2 pLPS) received na-CT alone. Median tumor size was 6.6 cm pre-NAT and 8.5 cm post-NAT. Median viable tumor was 100% pre-NAT and 60% post-NAT. 6 patients (1 DDLPS, 5 MRCLPS) received na-CT and na-RT. Median tumor size was 9.3 cm pre-NAT and 3.0 cm post-NAT. Median viable tumor was 90% pre-NAT and 2.5% post-NAT: DDLPS was 100% and 90% viable pre- and post-NAT; MRC was 100% and 2.5% viable pre- and post-NAT. 3 patients with MRC who received na-CT and na-RT had pathologic complete response (pCR). 1 MRC patient had PM and LR. 1 DDLPS patient had DR.

Conclusion: NAT was given to a higher proportion of patients with MRCLPS or pLPS. IA/IP DDLPS and MRCLPS tumors had the highest proportion of PM. NAT reduced the number of

patients with positive margins at resection across treatment modalities. In patients receiving na-RT alone, changes in tumor size did not reflect changes in tumor viability pre- and post-NAT. Patients who received both na-RT and na-CT had reductions in tumor size and viability. Na-RT alone or in combination with na-CT was associated with a decreased LR and DR while na-CT alone was associated with an increased LR and DR. MRCLPS patients treated with na-RT and na-CT had multiple pCRs and had fewer PM, LR, and DR compared with MRCLPS treated with either modality alone.

Table 1: Patient Characteristics

	Histology			
	All (79)	DDLPS (43)	MRCLPS (25)	pLPS (11)
Mean Age (SD)	57.0 (13.7)	62.9 (10.0)	45.9 (12.3)	60.6 (12.4)
Race				
White	72 (91%)	39 (91%)	24 (96%)	9 (82%)
Black	5 (6.3%)	4 (9.3%)	0 (0%)	1 (9.1%)
Other/Unknown	2 (2.5%)	0 (0%)	1 (4.0%)	1 (9.1%)
Sex				
Male (%)	48 (61%)	27 (63%)	13 (50%)	8 (72.7%)
Female (%)	31 (39%)	16 (37%)	13 (50%)	3 (27.3%)
Location (% PM)				
UE/LE	39 (11%)	10 (50%)	23 (13%)	6 (17%)
IA/P	38 (85%)	33 (70%)	3 (33%)	2 (0%)
CW/AW	2 (0%)	0 (0%)	0	2 (0%)
RN	1 (100%)	0 (0%)	0	1 (100%)
Treatment (% of pts)				
No NAT	40 (50%)	27 (63%)	9 (36%)	4 (36%)
na-RT	27 (34%)	13 (30%)	9 (36%)	5 (45%)
na-CT	6 (7.5%)	2 (4.7%)	2 (8%)	2 (18%)
na-RT & na-CT	6 (7.5%)	1 (2.3%)	5 (20%)	0 (0%)
Outcomes (% of pts)				
PM	34 (43%)	28 (65%)	4 (15%)	2 (18%)
LR	34 (43%)	26 (60.5%)	6 (25%)	2 (18%)
DR	29 (36%)	17 (39.5%)	5 (19%)	7 (64%)

Table 2: Tumor Characteristics and Recurrence based on Treatment Modality

Pt Treatment (n)	pre-NAT size cm (r)	post-NAT size cm (r)	pre-NAT viability % (r)	post-NAT viability % (r)	PM (% of pt)	LR (% of pt)	DR (% of pt)
No NAT (40)	11.8 (2.5-53)	NA	95 (10 -100)	NA	22 (55%)	19 (44.2%)	15 (34.9%)
na-RT (27)	15.5 (5.6-34)	15.8 (5.1-45.6)	100 (80-100)	45 (1-100)	9 (33%)	10 (37%)	11 (40.7%)
na-CT (6)	6.6 (2.2-13.8)	8.5 (2.3-16.4)	100 (100-100)	80 (0-95)	2 (33.3%)	4 (80.0%)	4 (80.0%)
na-RT & na-CT (6)	9.3 (6.1-25.2)	3.0 (0-15.3)	100 (80-100)	2.5 (0-90)	1 (16.7%)	1 (16.7%)	1 (16.7%)

Table 3: MRCLPS Tumor Characteristics and Recurrence based on Treatment Modality

MRCLPS Treatment (n)	pre-NAT size cm (r)	post-NAT size cm (r)	pre-NAT viability % (r)	post-NAT viability % (r)	PM (% of pt)	LR (% of pt)	DR (% of pt)
no NAT (9)	9 (4.7-21)	NA	95 (60-100)	NA	1 (11%)	1 (11%)	0 (0%)
na-RT (9)	14.7 (5.6-20)	13 (5.1-21.6)	100 (100)	10 (2-90)	1 (11%)	2 (22%)	4 (44%)
na-CT (2)	6.1 (2.2-10)	7.6 (2.3-13)	100 (100)	60 (0-80)	1 (50%)	1 (50%)	1 (50%)
na-RT & na-CT (5)	8.6 (6.1-25.2)	0 (0-15.3)	100 (80-100)	0 (0-40)	1 (20%)	1 (20%)	0 (0%)

(P 219) ELECTIVE DISCONTINUATION OF SORAFENIB TREATMENT IN DESMOID TUMORS: A RETROSPECTIVE ANALYSIS WITH IMPLICATIONS FOR CLINICAL PRACTICE

Nikita Shah, M.D.[1]; Ayra Faruqi, MD[2]; Borislav Alexiev, M.D.[3]; John Hayes, M.D.[3]; Susan Abbinanti, M.D.[3]; Fahad Faruqi, M.D., M.P.H[2]; Akhil Chawla, MD[3]; Terrance Peabody, M.D.[3]; Samer Attar, M.D.[3]; Jeff Wayne, M.D.[3]; Seth Pollack, MD[4]; Pedro Viveiros, MD[5]; Vinai Gondi, M.D.[3], (1) Northwestern Memorial Hospital, Lithia, FL, (2) Northwestern Medicine, Warrenville, IL, (3) Northwestern Memorial Hospital, Chicago, IL, (4) Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL, (5) Northwestern University, Chicago, IL

Objective: Desmoid tumors are potentially serious, painful and debilitating but non-cancerous growths with high rates of local recurrence after surgery. Treatment decisions can be complex as these tumors will often remain dormant or even spontaneously regress. Recent studies have challenged the central role of surgery in desmoid management, and many institutions will now start with active surveillance for many asymptomatic patients followed by systemic treatment when needed. In patients with symptomatic or progressive desmoid tumors, sorafenib was associated with improved sustained responses and long-term disease control compared to placebo. However, the optimal duration of treatment is not well-established and many clinicians may be reluctant to stop sorafenib in patients with responsive desmoid tumors, though sorafenib may have significant toxicity impacting quality of life.

Here we present a retrospective analysis of patients at a single institution who were treated with sorafenib and obtained disease control. These patients were followed during and after the discontinuation of treatment.

Methods: This study was a retrospective analysis of desmoid tumor patients seen at a high-volume, multidisciplinary sarcoma program from January 2021 to April, 2023. This was a retrospective review of a prospectively collected clinical database and was part of an IRB approved study. Clinical variables included whether or not patients received sorafenib, the duration of treatment, change in tumor size during treatment, reason for discontinuation and clinical outcomes after stopping treatment.

Results: Out of a total forty-six patients seen during this time period, nineteen were treated with sorafenib. All nineteen patients treated with sorafenib in this study are still alive.

One patient progressed on sorafenib. This patient, who has an aggressive tumor with an APC mutation, has now been on over ten different systemic treatment regimens and has been through multiple types of locally directed therapies. Out of the other eighteen patients treated with sorafenib, thirteen patients electively discontinued sorafenib (72%) in order to improve their quality of life and five patients are still undergoing treatment.

The average time on sorafenib prior to discontinuing the drug was 13.05 weeks, range (2-54 weeks) and standard deviation 13.74 weeks. After discontinuing sorafenib, the average length of follow-up was 21.78 months, range (7-49 months) and a standard deviation 15.75 months.

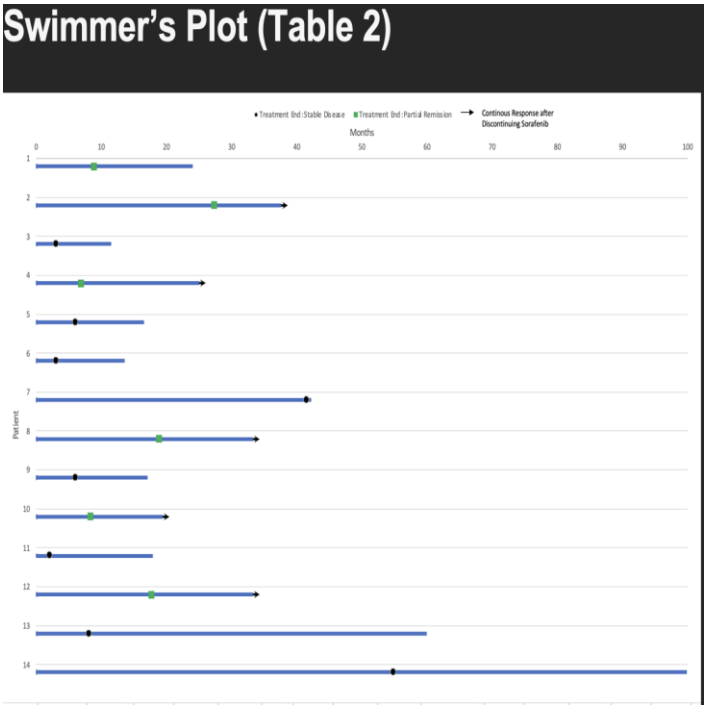
After electively stopping treatment, 53.8% of patients had stabilization of their desmoid tumor. Following discontinuation of sorafenib, 38.5% had continuous response to the drug.

Notably, one patient (7.7%) had progression of their desmoid tumor after discontinuation of sorafenib. Because this patient was followed closely, they were immediately put back on sorafenib and the patient's disease was then stabilized.

Conclusion: The data suggests that it is safe to discontinue sorafenib for patients with desmoid tumors after a limited treatment course when there is close observation. However, it is important to recognize that one patient did have progressive disease after treatment discontinuation. In the present study the one who progressed off treatment, regained control over their disease after sorafenib was reintroduced. This emphasizes the importance of active surveillance following discontinuation of oral therapy. Further research is needed to establish the optimal treatment duration of sorafenib for patients with desmoid tumors. Collaborative efforts between institutions can provide valuable insight into patient outcomes and help guide future treatment strategies contemplating holding sorafenib.

Demographics of Desmoid Patients Treated with Sorafenib (Table 1)

	Sorafenib (n=14)
Age	33.29
Gender (F)	42.9% (6)
Tumor Location (IntraAbdominal)	57.1% (8)
Size of Tumor (> 10 cm at Dx)	71.4% (10)
Radiation History	14.3% (2)
Surgery History	14.3% (2)
Pregnancy History	7.1% (1)



Novel Diagnostics and Bioinfomatics

(P 220) **[68GA]GA-FAPI PET/CT AS NOVEL DIAGNOSTIC TOOL IN SARCOMA**

Rainer Hamacher, MD[1]; Helena Lanzafame, MD[2]; Ilektra A. Mavroeidi, MD[1]; Kim M. Pabst, MD[2]; Nader Hirmas, MD[2]; Lukas Kessler, MD[2]; Sebastian Bauer, MD[1]; Lars-Erik Podleska, MD[3]; Ken Herrmann, MD[2]; Jens T. Siveke, MD[4]; Wolfgang P. Fendler, MD[2], (1) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (2) Department of Nuclear Medicine, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (3) Department of Musculoskeletal Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (4) Bridge Institute of Experimental Tumor Therapy, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany

Objective: Radiolabeled fibroblast activation protein (FAP) inhibitors (68Ga-FAPI-46) show high tumor uptake on PET in several tumor entities and therefore represent a promising novel molecular imaging method in oncology besides 18F-FDG PET. This is particularly interesting for bone sarcomas (BS) and soft-tissue sarcomas (STS) that demonstrate high expression of FAP on tumor cells and associated fibroblasts. Here we aimed to investigate the diagnostic performance of FAP-directed 68Ga-FAPI PET/CT in BS and STS and compared it to 18FDG PET/CT.

Methods: Patients with BS or STS undergoing clinical 68Ga-FAPI PET for staging/restaging

were eligible for enrollment into the 68Ga-FAPI PET prospective observational trial. A group of patients also underwent 18F-FDG PET. Number of detected lesions and tracer uptake (SUVmax) for T, N and M were analyzed. Non-parametrical t-test was employed for semiquantitative analysis. Association between 68Ga-FAPI PET uptake and histopathologic FAP expression was analyzed with Spearman r correlation.

Results: We included 200 sarcoma patients (65 BS, 135 STS), of which 32 (16%) were low, 141 (71%) were high-grade sarcomas and 27 (14%) sarcoma entities for which grading doesn't apply (N/A). 68Ga-FAPI vs. 18F-FDG PET detected a total of 1174 (81%) vs. 1023 (71.5%) lesions. On a per-region basis, 68Ga-FAPI vs. 18F-FDG PET demonstrated higher detection efficacy for T (144 (100%) vs. 124 (86%) lesions) and M (1167 (97%) vs. 1026 (86%) lesions). Moreover, we observed as well a higher sensitivity (96% vs. 88%), specificity (88% vs. 60%), PPV (99% vs. 95%), NPV (68% vs. 38%) and overall accuracy (95% vs. 88%) for 68Ga-FAPI when compared to 18F-FDG. Radiotracer uptake (SUVmax) was significantly higher for 68Ga-FAPI vs. 18F-FDG in low grade (10.4±8.5 vs. 7.1±4.5, p=0.01) and N/A (22.3±12.2 vs. 8.6±10.8, p=0.004) tumors as well as in patients with SFT (T: SUVmax 23.5±11.9 vs. 4.5±8.7, p=0.0005; M: SUVmax 17.±28.7 vs. 3.8±2.0, p=0.0001) and myxoid liposarcoma(T: SUVmax 5.6±12.2 vs. 3.5±2.1, p=0.04). The association of 68Ga-FAPI uptake and histopathologic FAP expression (n = 89) showed a moderate positive correlation (Spearman r = 0.43; p < 0.0002).

Conclusion: 68Ga-FAPI PET/CT is as novel imaging tool for sarcoma and shows superiority over 18F-FDG PET/CT particularly in low grade sarcoma.

Table: Patient and tumor characteristics

Characteristics	Median[IQR]	N	(%)
Age(y)	55 [39-65]		
Sex			
Female		91	(45)
Male		109	(55)
Indication			
Staging		49	(25)
Restaging		151	(75)
Sarcoma type			
Bone sarcoma		65	(33)
Osteosarcoma		17	(9)
Chondrosarcoma		17	(9)
Other BS		15	(7)
Ewing		8	(4)
Spindle cell sarcoma		5	(3)
UPS		3	(1)
Soft Tissue sarcoma		135	(67)
Other STS		41	(21)
SFT		22	(12)
UPS		15	(7)
Dedifferentiated liposarcoma		15	(7)
Myxoid liposarcoma		14	(6)
Leiomyosarcoma		14	(6)
Synovial sarcoma		7	(4)
Spindle cell sarcoma		7	(4)
Grading			
N/A		27	(14)
Low		32	(16)
High		141	(70)
Staging at time of diagnosis			
Localized		109	(55)
Metastasized		91	(45)
Site of metastases at time of diagnosis			
Lung		82	(90)
Bone		33	(36)
Lymph nodes		27	(30)
Liver		24	(26)
Visceral		18	(20)
Prior Therapies			
Surgery/local resection		142	(90)
Radiation Therapy		77	(49)
Chemotherapy		97	(62)
Hormone therapy		2	(1)
Immune therapy		9	(5)
Other (e.g. tyrosine kinase inhibitors, cdk4/6 inhibitors)		6	(4)
None		42	(22)
Intented treatment before PET			
Surgery/local resection		74	(44)
Radiation Therapy		48	(29)
Chemotherapy		113	(68)
Hormone therapy		4	(2)
Immune therapy		6	(4)
Radionuclide therapy		9	(5)
Bone targeted therapy		1	(1)
Active surveillance		24	(14)
Other		33	(20)
Implemented planned treatment			
Yes		155	(78)
No		45	(22)

(P 221) EARLY RESULTS IN NOVEL USE OF CONTRAST-ENHANCED SUSCEPTIBILITY-WEIGHTED IMAGING IN THE ASSESSMENT OF RESPONSE AND PROGRESSION IN DESMOID FIBROMATOSIS: PILOT STUDY IN A SPECIALIZED CANCER INSTITUTION

Raul F. Valenzuela, M.D[1]; Behrang Amini, M.D, PhD[2]; Elvis Duran Sierra, PhD[1]; Mathew Antony, M.D[3]; Colleen Costelloe, M.D[3]; John E. Madewell, M.D[1]; William A. Murphy, Jr., M.D[4], (1) MD Anderson Cancer Center, Houston, TX, (2) MD Anderson cancer Center, Houston, TX, (3) MD Anderson cancer center, Houston, TX, (4) M.D Anderson cancer center, Houston, TX

Objective: To provide initial evidence outlining the novel use of CE-SWI as a single MRI sequence capable of providing insight regarding the underlying biological changes of responding and progressing desmoid tumors using an improved tridimensional volume assessment allowing improved separation of T2 hypointense collagenized mature tumor from T2 hyperintense, T1 shortened enhancing, immature, progressive tumor components.

Methods: An IRB-approved waiver of consent was obtained for this retrospective study, including an initial pilot analysis of ten single-lesion extremity desmoid fibromatosis patients undergoing standard-of-care MRI examinations in our institution. The scans included advanced imaging sequences, including diffusion-weighted imaging (DWI/ADC), perfusion-weighted imaging with dynamic contrast enhancement (PWI/DCE), and contrast-enhanced susceptibility-weighted imaging (CE-SWI), performed between March 2021 and May 2023.
CE-SWI and T2-STIR data was collected from each patient across multiple time points in their treatment. A three-dimensional manual tumor segmentation was performed in 48 VOIs by an imaging-specialized research assistant and a 27-year-experienced skeletal radiologist using MIM commercial software.

Data obtained from each VOI included maximum diameter, volume, and modified Choi (mChoi) measurements from CE-SWI and T2-STIR sequences. In-house developed software (CARPI-AF: Cancer Radiomic and Perfusion Imaging Automated Framework) was used to automatically extract five first-order radiomic features (mean, skewness, kurtosis, 10th and 90th percentiles) from the SWI and T2 images. Patient response was assessed using the conventional Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 as a reference standard and compared against T2-STIR and CE-SWI volumetric, mChoi, first-order radiomic features, and routine radiologic reporting (RRR). Thresholds for progression and response were set at 20% and 30% for unidimensional RECIST and volumetric mChoi assessments. An increase of 25% and a decrease of 50% were considered thresholds for progression and response, respectively, for 3-D volumetric assessment. mChoi values were estimated as a normalization quotient obtained by dividing an entire desmoid lesion's mean 3D intensity value by the adjacent standard muscle intensity value.

Results: This study included eight female and two male patients with an average age of 42 years (range 19-61 years). Five patients were treated with Sorafenib, two with Pazopanib, and three were undergoing active surveillance without therapy. Of the ten patients, two (2) were considered true progression by T2-based RECIST, while the other eight (8) were considered stable. No true T2-RECIST-based positive responses were included in the pilot study.

True T2-based RECIST progression: Two patients displayed true progression by the T2-based RECIST reference standard. This same result was detected by CE-SWI volume and mChoi at an average of 4.5 months earlier than T2-based RECIST. Both cases were assessed by routine radiologic reporting (RRR) as progression, at the same time as CE-SWI volume and mChoi. In true progression cases, SWI detected an average of 23% more voxels within the 90th

percentile relative to T2, while T2 demonstrated an average of 8.5% more voxels within the 10th percentile than SWI.

T2-based RECIST Stability:
RECIST Stable with discrepant progression: Four patients presented as stable using T2-based RECIST. Progression was determined in these patients by an increase greater than 25% of both T2-based volume (in three patients) and CE-SWI-based volume (in two patients). Routine radiologic reporting (RRR) determined only one of the four to be progression.

RECIST stable with a discrepant positive response: Four patients displayed stable T2-based RECIST with discrepant positive response predicted by CE-SWI mChoi (in three patients) and T2 mChoi (in three patients). All cases assessed by mChoi as responding patients were also considered positive responses by routine radiology reporting (RRR).

Conclusion: This pilot study suggests that radiologists (RRR) assessing desmoid tumors over time are relatively insensitive to volumetric tumor changes that precede RECIST detectable progression. While, they tend to be better tuned with changes in T2 hypo intensity as a measure of collagenization and T1 shortening-enhancement as a measure of progressive disease, which is manifested in parallel with the mChoi values, with respect to both positive mChoi responses in stable RECIST and to mChoi progression in RECIST progression.
All eight cases of T2-RECIST stability (100%) had a discrepant evaluation of either progression by volume or positive response by mChoi, suggestive of the insensitivity of RECIST to detect the biologic changes displayed by desmoid tumors undergoing systemic treatment or active surveillance.
Trends, including a higher % of hyperintense voxel capture (90 percentile) and earlier detection of response by mChoi, suggest an overall performance advantage of CE-SWI volumetric and mChoi assessments over conventional T2-weighted equivalents and traditional RECIST.
The expected trend of increasing 10th percentile hypointense voxels, decreasing 90th percentile hyperintense voxels, decreasing mean, or increasing skewness in responding patients, and the opposite in progressive disease, was present at least in one of the four features [Table 1] in nine patients (90%), while all four combined, only in three (30%). Such results align with the statistical power expected from a small sample, outlining the need for an extension to a larger population analysis. Our early results suggest that the novel use of CE-SWI-based volumetric and mChoi measurements could improve the prediction of response/progression in desmoid tumors by providing a better assessment of multi-dimensional tumor size and increased discrimination between the collagenized mature component and the enhancing immature components, respectively predominant in mature-responding and immature-progressive disease.

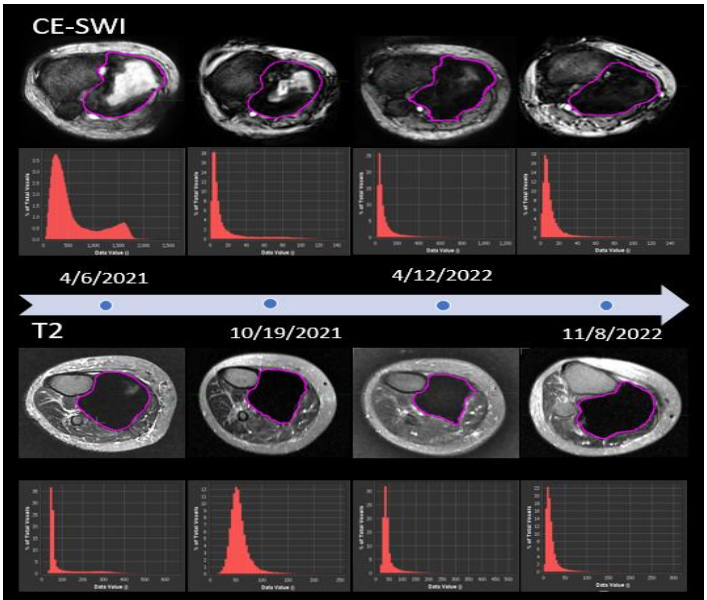


Figure 1. Comparison between CE-SWI and T2 images and their respective intensity histograms of a desmoid fibromatosis across 4-time points undergoing response to treatment from left to right demonstrating the process of reduced enhancement and increase T2 hypointensity during response and collagenization.

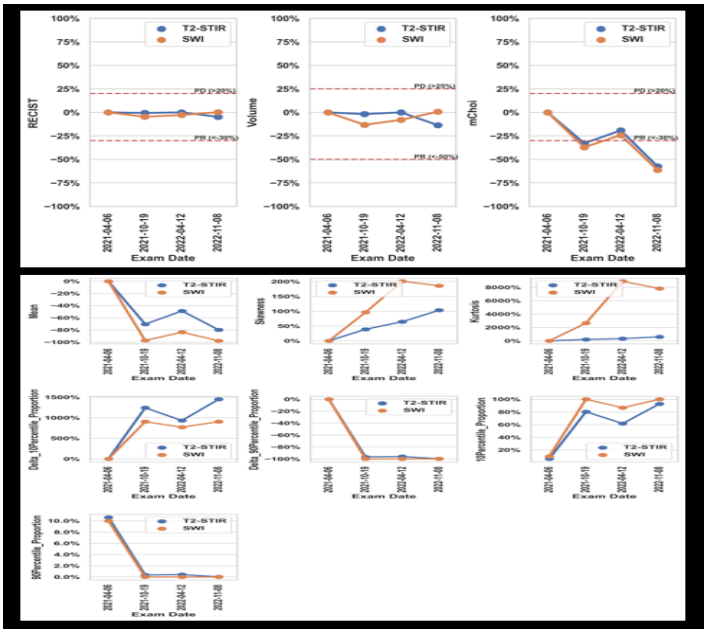


Figure 2. Point plots of RECIST, Volume, mChoi, and first-order radiomic features demonstrating expected traits of response including decreased mean, increased skewness, increased 10th percentile, and decreased 90th percentile.

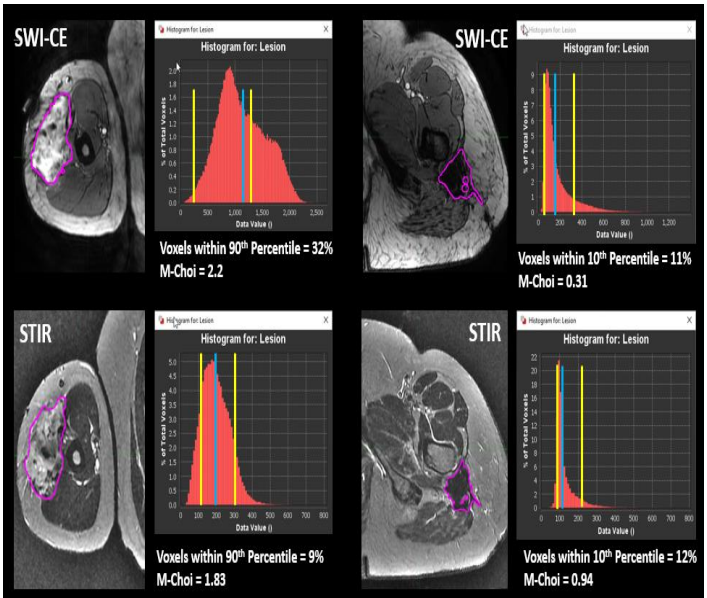


Figure 3. Left panels show a higher percentage of voxels within the 90 percentile in SWI-CE versus T2 in an active progressing lesion. Right panels show a higher percentage of voxels within the 10 percentile in T2 versus SWI-CE in a highly collagenized responding tumor. SWI-CE imaging captures 23% more 90th percentile voxels than T2.

Table 1

	Progression by RECIST T2				Stable RECIST discrepancy with progression								Stable RECIST discrepancy with response							
	Case 1		Case 2		Case 3		Case 4		Case 6		Case 10		Case 5		Case 7		Case 8		Case 9	
Sequence	SWI	T2	SWI	T2	SWI	T2	SWI	T2	SWI	T2	SWI	T2	SWI	T2	SWI	T2	SWI	T2	SWI	T2
Mean	Yes	Yes	Yes	No	No	No	No	Yes	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No
Skewness	No	No	Yes	Yes	No	No	Yes	Yes	No	No	No	Yes	No	No	Yes	Yes	No	Yes	No	Yes
10%	Yes	Yes	Yes	No	No	No	No	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No
90%	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No
Total	3	3	4	2	0	0	1	3	3	3	0	2	0	1	4	4	3	4	0	1

- SWI detected at least 1 concordant pattern in 6/10 (60%) cases
- T2 detected at least 1 concordant pattern in 9/10 (90%) cases
- Cases with at least 1 concordant pattern detected: 9/10 (90%)
- Cases with all concordant patterns detected: 3/10 (30%)

Progression:
Mean Goes up
Skewness goes down
10% goes down
90% goes up

Response:
Mean Goes down
Skewness goes up
10% goes up
90% goes down

(P 222) EXPLORING THE IMPACT OF NGS ON DIAGNOSTICS AND TREATMENT OF SARCOMA: INSIGHTS FROM REAL-WORLD DATA ACROSS MULTIPLE INSTITUTIONS IN EUROPE

ANASTASIOS KYRIAZOGLOU, MD,PhD[1]; Agnieszka Zajac, MD,PhD[2]; Claudia Valverde, MD[3]; Anna M. Czarnecka, MD PhD[4]; Piotr Blonski[4]; Cleofé Romagosa, MD[5]; Katarzyna Seliga, MD[6]; Asimina Koulouridi, MD[7]; Anastasia Mala, N/A, MD[7]; Michal Wagrodzki, MD, PhD[8]; Tulio M. Silva, MD[9]; Marcin Witczak, MSc[10]; Iwona Ługowska, n/a[11]; Magdalena Rosińska, n/a[12]; Rosa Álvarez Álvarez, PhD, MD[13]; Marta Aguirre, MD[14]; Andrzej Tysarowski, MD[15]; Stefania Kokkali, N/A, MD[16]; Vasileios Georgoulias, N/A, MD,PhD[17]; César Serrano, MD, PhD[18]; Piotr Rutkowski, MD, PhD[19]; Ioannis Boukovinas, N/A, MD,PhD[20], (1) Attikon University Hospital, ATHENS, AEGALEO, Attiki, Greece, (2) Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland, Warsaw, Mazowieckie, Poland, (3) Vall D'Hebron University Hospital, Barcelona, Catalonia, Spain, (4) Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (5) Pathology Department, Valld'Hebron University Hospital, Barcelona, Spain, barcelona, Catalonia, Spain, (6) Cancer Molecular and Genetic Diagnostics Department, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland, Warsow, Podlaskie, Poland, (7) Department of Oncology, University Hospital of Heraklion, Crete, Greece, Iraklion, Iraklion, Greece, (8) Maria Sklodowska-Curie National Research Institute of Pathology, Warsaw Poland, Warsaw, Mazowieckie, Poland, (9) Hospital Universitario Vall d'Hebron, Barcelona, Catalonia, Spain, (10) Early Phase Clinical Trials Unit, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland, Warsaw, Mazowieckie, Poland, (11) Early Phase Clinical Trials Unit, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland., Warsaw, Mazowieckie, Poland, (12) Department of Computational Oncology, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland., Warsaw, Mazowieckie, Poland, (13) Medical Oncology Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain., Madrid, Madrid, Spain, (14) Department of Oncological Mathematics, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland, Warsow, Podlaskie, Poland, (15) Early Phase Clinical Trials Unit, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland, Warsow, Podlaskie, Poland, (16) National and Kapodistrian University of Athens, Oncology Unit, 2nd Department of Medicine, HIPPOKRATIO General Hospital of Athens, Athens, Attiki, Greece, (17) National network of precision medicine, Greece, athens, Attiki, Greece, (18) Medical Oncology Department, Hospital Universitario Vall d'Hebron, Vall d'Hebron Institute of Oncology, Barcelona, Spain, Barcelona, Catalonia, Spain, (19) Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Mazowieckie, Poland, (20) Department of Medical Oncology, Bioclinic Hospital, 54622 Thessaloniki, Greece, Thessaloniki, Thessaloniki, Greece

Objective: The role of next-generation sequencing (NGS) is still a matter of debate in patients with sarcoma, given the biological peculiarities such as the low mutational burden and the lack of clonal, recurrent, and/or targetable alterations in most of the sarcoma entities. This retrospective, international, and multicenter study aimed to evaluate the clinical importance of NGS for diagnosis and therapeutics of sarcomas in a real-world European scenario.
The primary focus of this investigation was on the application of NGS technologies in the context of subtypes of sarcoma, with specific attention given to their diagnostic utility. Subsequently, the impact of NGS on diagnostic assessment was thoroughly examined. Secondly, this study was also designed to elucidate the usefulness of NGS for identifying actionable mutations in patients with sarcoma, thus improving the prospects of targeted

therapeutic interventions in the field of sarcoma therapeutics, which remains controversial. The main objectives of the current study are to determine/quantify the percentage of patients who share specific mutations, the exact subtypes of sarcomas in which targetable mutations are detected and the clinical benefit of those who received targeted therapy.

Methods: Five sarcoma expert institutions from Greece (n=2), Poland (n=1), and Spain (n = 2) participated in this study to reflect the inherent variability between countries and between institutions. DNA and RNA for NGS from bone and soft tissue sarcomas were isolated from formalin-fixed paraffin-embedded tumor tissues. The NGS test was selected at the discretion of the referring physicians in each center. Several diagnostic and/or therapeutic NGS panels were used in all centers participating in this study, including FoundationOneCDx, FusionPlex Sarcoma and Sarcoma v2 panels, and FusionPlex Comprehensive Thyroid and Lung Kit. The relevant patients' demographic and clinical data were recovered from the medical records for descriptive purposes and for correlation with the NGS results. All statistical analyses were performed using R version 4.2.3.

Results: We analyzed a total of 696 patients. The cohort's median age was 47 years (0-89). 379 (54.4%) of the cases were male, and 308 (44.2%) were female. NGS was performed predominantly at the localized stage of the disease (n=406, 58.3%) rather than in metastatic cases (n=267, 38.4%) (Table 1). The most common subtypes that underwent NGS were leiomyosarcomas (n=62), Ewing sarcomas (n=53), and undifferentiated pleomorphic sarcomas (n=51 cases) (Table 2).
Genetic aberrations were found in nearly half of the cohort (n=333, 47.8%). In total, 521 genetic alterations of any type were found in these 333 patients: 274 (52.6%) were mutations, 175 (33.6%) were fusions and 72 (13.8%) gene amplifications. The most frequent findings were as follows: mutations affecting TP53 (n=40), RB1(n = 13) and PIK3CA (n=11); fusions involved EWSR1-FLI1 (n=20), NAB2-STAT6 (n=15), and CIC-DUX4 (n=12); and amplifications of MDM2 (n=9) and CDK4 (n=8).
For the 510 cases in which NGS was performed for diagnostic purposes, 63 (9.1%) resulted in a change from the original diagnosis based on the genetic testing results. CIC-rearranged sarcomas (n=7) and solitary fibrous tumor (n=5) were the most common new diagnoses, followed by PEComas (n=4), Ewing sarcomas (n=4),NTRK-rearranged sarcomas (n=3), and clear cell sarcomas(n = 3).(Table 3).
Ofthe 333 patients with at least one genetic alteration found in the NGS analyses, 136 cases (41% and 19.5% of the entire cohort) had potentially actionable mutations. However, therapies could be administered only in 17 patients, representing 13% of these patients and 2.4% of the entire cohort (Table 4).
Targetable mutations with relevant therapies were detected in 136 cases (19.5%), with only 17 patients (2.4%) receiving targeted therapy.

Conclusion: Various NGS technologies and platforms are increasingly used in oncology centers, primarily for therapeutic indications. Unlike most cancer types, the indication of NGS to provide some aid in diagnosis is paradigmatic in the case of sarcomas, given the pleiad and complexity of the histotypes of sarcomas. On the other hand, the therapeutic options for patients with sarcoma are limited, and NGS testing offers the promise of finding targetable alterations. However, this has been explored mostly in comprehensive cancer centers in the United States, and a clear picture of the more complex reality in Europe is lacking.
Our retrospective study shows that molecular NGS testing can help diagnose sarcomas only in a small proportion of cases (9%). Most of the changed diagnoses could only be made with molecular techniques, as they involved gene rearrangements. Therefore, the prior review of the cases by sarcoma-expert pathologists was key to the need for NGS in this setting.
Our series agrees with previous work that showed a low number of genetic alterations found

with NGS, with only half of the patients here harboring at least one. Furthermore, the current study reveals interesting data on the therapeutic impact of NGS in sarcomas. Targetable mutations were detected in 19.5% (136) of patients. However, only 2.4% (17) received appropriate targeted therapy. Thus, the difficulty in obtaining off-label use of alternative drugs significantly limits the potential impact of NGS on the therapeutics and outcomes of sarcomas. Due to the heterogeneity of the population included, it is difficult to conclude the relevance of specific targetable mutations in specific subtypes of sarcomas.
Our results encourage the future generation of an international database collecting actionable NGS mutations detected in sarcoma patients to understand their role in specific subtypes better.

Total number	**696**
Sex:	
Male	379 (54.5%)
Female	308 (44.2%)
Unknown	9 (1.3%)
Age: range (median)	0-89 (47) years
Disease stage at the time of NGS:	
Localized	406 (58.3%)
Metastatic	267 (38.4%)
Unknown	23 (3.3%)

Table 1. Demographic and clinical characteristics of the patients.

Sarcoma histotype	**Number of patients**
Leiomyosarcoma	62
Ewing sarcoma	53
Undifferentiated pleomorphic sarcoma	51
Undifferentiated sarcoma NOS	36
Myxofibrosarcoma	32
Undifferentiated spindle cell sarcoma	26
Synovial sarcoma	24
Malignant peripheral nerve sheath tumour	23
Endometrial stromal sarcoma	20
Chondrosarcoma NOS	19
Dedifferentiated liposarcoma	19

Table 2. The most frequent sarcoma subtypes.

New diagnosis established after NGS	**Number of patients**
CIC-rearranged sarcoma	7
Solitary Fibrous Tumour, malignant	5
Ewing sarcoma	4
PEComa	4
Clear cell sarcoma	3
NTRK-rearranged spindle cell neoplasm	3
Undifferentiated round cell sarcoma	3
Undifferentiated spindle cell sarcoma	3
Chondrosarcoma	2
Dedifferentiated liposarcoma	2
Desmoplastic small round cell tumour	2
Dermatofibrosarcoma protuberans	2
Extraskeletal myxoid chondrosarcoma	2
Myxofibrosarcoma	2
Ossifying fibromyxoid tumour, malignant	2
Sclerosing epithelioid fibrosarcoma	2

Table 3. The most frequent new diagnoses, established according to the NGS result.

	Genetic aberrations found with NGS	**Agent name**	**Duration of the treatment [months]**
1	RAD50, BRCA2	Olaparib	2,5
2	KRAS, TP53, CHEK2, KDR, ALK	Olaparib	1
3	BRAF, PGFR1, ATM	Dabrafenib/Trametinib	12
4	ALK	Crizotinib	20
5	MET amplification	Cabozantinib	5
6	MET amplification	Capmatinib	1
7	FANCD2, AR amplification	Bicalutamide	3
8	FANCD2, AR amplification	Olaparib	3
9	NF1	Trametinib/everolimus	3
10	IDH1, KRAS	Olaparib	0,75
11	EWSR1 - ATF1 fusion	Pazopanib	3
12	EWSR1 - TFCP2 fusion, LOC101929458 – ALK fusion	Alectinib	4
13	BRAF	Encorafenib + binimetinib	19
14	TP53	Sirolimus	6
15	Amplification of CDK4 MDM2, HMGA2	Milademetan	2
16	SPECC1L - NTRK3 fusion	Larotrectinib	2,5
17	IDH1	CID#305432101	6

Table 4. Targeted therapy regimens administered according to the NGS result.

(P 223) SIGNATERA CTDNA TRACKER IN SARCOMA: CLINICAL EXPERIENCE

Adrienne Victor, MD, University of Rochester, Rochester, NY

Objective: Circulating tumor DNA (ctDNA) holds promise as a biomarker for disease burden and risk profiling in all cancers, and may be particularly valuable in sarcomas which lack circulating tumor markers. Use of ctDNA as a clinical tool has been limited, with most data confined to a research-use-only setting. He we report our institutional experience with the signatera assay, a commercial test tracking 16 tumor-specific, clonal, somatic, single nucleotide variants (SNVs) and reported as a number of mean tumor molecules per milliliter (MTM/ml).

Methods: Signatera results on all sarcoma patients at the University of Rochester were reviewed and compared with clinical outcomes.

Results: Thirty-two patients with sarcoma had signatera testing completed. Six tests failed, usually due to insufficient remaining tissue for sequencing. Twenty-six had results, of which 13 were positive and 13 negative. The average time from having all materials (plasma drawn + tumor received for sequencing) to first result was 19 days (range 11-37). The average time from the second draw to result was 8 days (range 5-18). For those with metastatic disease at time of testing, 10 were positive and 4 were negative. For those without known metastatic disease, 3 were positive and 8 were negative. One patient had an initial very low positive test (0.04 MTM/ml) after finishing curative intent treatment that was subsequently negative without additional intervention. Three patients with measurable disease (all metastatic) were ctDNA negative vs 11 with measurable disease (8 metastatic) had detectable ctDNA.

Conclusion: Signatera was positive in 79% (11/14) of sarcoma patients with clinically detectable disease and 17% (2/12) of patients who were no evidence of disease (NED) at evaluation prior to testing. One of these appeared to be a “false positive” while the other is pending restaging scans. This test may be particularly valuable when imaging is indeterminant. The lead time to first result may make it difficult to utilize this test in risk stratifying for neoadjuvant therapy, but could be feasible in the adjuvant setting. Criteria for response, progression, and optimal frequency of testing requires further study. The need for tissue and sequencing solely for the signatera assay is a potential limitation.

(P 224) CO-MUTATION-DEPENDENT PROGNOSIS IN PATIENTS WITH ADVANCED SOFT TISSUE SARCOMA

Maggie Y. Zhou, MD[1]; Minggui Pan, MD[2]; Chen Jiang, PhD[3]; Zheyang Zhang, MS[4]; Nam Bui, MD[5]; Jeffrey Bien, MD[6]; Amanda Siy[7]; Ninah Achacoso, MS[3]; Aleyda Solorzano, MD[3]; Pamela Tse, BA[3]; Elaine Chung, MPH[3]; Sachdev Thomas, MD[8]; Laurel Habel, PhD[3]; Kristen N. Ganjoo, MD[9], (1) Stanford University School of Medicine, Palo Alto, CA, (2) Stanford University, Palo Alto, CA, (3) Kaiser Permanente, Oakland, CA, (4) Xiamen University, Xiamen, Fujian, China (People's Republic), (5) Stanford University, Stanford, CA, (6) Stanford University School of Medicine, Stanford, CA, (7) Stanford University School of Medicine, El Dorado Hills, CA, (8) Kaiser Permanente, Vallejo, CA, (9) Stanford Cancer Center, Palo Alto, CA

Objective: To examine the impact of the most common genomic alterations TP53, CDKN2A, RB1, PTEN, and ATRX mutations on prognosis of advanced soft tissue sarcoma (STS).

Methods: This cohort included patients from Kaiser Permanente Northern California and Stanford Cancer Center with grade 2 or higher locally advanced and metastatic STS with next-generation sequencing performed. We used Cox regression modeling to examine the association between the individual mutations and overall survival (OS), adjusting for demographics, performance status, Charlson comorbidity index, receipt of systemic therapy, histology subtypes, and co-mutations.

Results: Of 602 total eligible patients, 28.9% had leiomyosarcoma (LMS), 22.6% had undifferentiated pleomorphic sarcoma (UPS), 13.0% had liposarcoma (LS), and 35.5% had other histology subtypes (Others). Among all patients, 49.7% had TP53 mutation (mutTP53), 23.1% had CDKN2A mutation (mutCDKN2A), 21.8% had RB1 mutation (mutRB1), 15.0% had ATRX mutation (mutATRX), and 9.5% had PTEN mutation (mutPTEN). Compared to LMS, OS of UPS and Others was worse (HR = 1.53, [95% CI 1.11-2.14] and HR = 1.52, [95% CI 1.10-2.10]) while OS of LS was similar (HR = 1.22, [95% CI .78-1.91]). MutPTEN was associated with worse OS compared to wild-type PTEN (wtPTEN, HR = 1.58, [95% CI 1.11 – 2.23]) and this association was preserved across different molecular subgroups. Worse OS with mutPTEN versus wtPTEN was much stronger in patients with wtTP53 (HR = 3.97, [95% CI 1.85 – 8.52]) and less strong in patients with mutTP53 (HR = 1.27, [95% CI .85 – 1.91]). Similarly, worse OS associated with mutCDKN2A versus wtCDKN2A was primarily driven by patients with wtTP53 (HR = 1.50, [95% CI 1.01 – 2.23]) but not patients with mutTP53 (HR = 1.00, [95% CI .60 – 1.60]). We also found that mutPTEN and mutRB1 appeared to be cooperative in driving adverse OS effect.

Conclusion: Prognosis of advanced STS appears to be histologic subtype and co-mutation-dependent. TP53 mutations appear to attenuate the adverse OS effect by mutPTEN and mutCDKN2A. Our results provide new insight that could be helpful for prognostic stratification in clinical practice and clinical trials and for understanding further the molecular mechanisms of STS.

(P 225) PAN-SARCOMA DATABASE (PSDB): A MODEL-VIEW-CONTROLLER FRAMEWORK UTILIZING REDCAP, SHINY, AND R TO CREATE A CONTINUOUS PIPELINE OF SARCOMA DATA

Brandon E. Rose, MD, MPH[1]; Priscila Barreto coelho, MD[2]; Steven Bialick, DO[3]; Pooja Patel[4]; Kurt Statz-Geary, n/a[4]; Osvaldo Nunez, Jr.[4]; Alina Kang[4]; Mason Thornton[4]; Aditi Dhir, MD[4]; Emily Jonczak, MD[5]; Gina Z. D'Amato, MD[6]; Jonathan Trent, MD[5], (1) University of Miami and Jackson Health Systems, Miami Beach, FL, (2) University of Miami, Miami, FL, (3) Sylvester Comprehensive Cancer Center, University of Miami, Deerfield Beach, FL, (4) University of Miami Miller School of Medicine, Miami, FL, (5) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL, (6) Sylvester Comprehensive Cancer Center, Miami, FL

Objective: The Pan-Sarcoma Database (PSDB) is an innovative open-source research application developed to efficiently collect and manage data on sarcoma patients to create a reliable research pipeline. Our data process uses free software, REDCap, to collect, update,

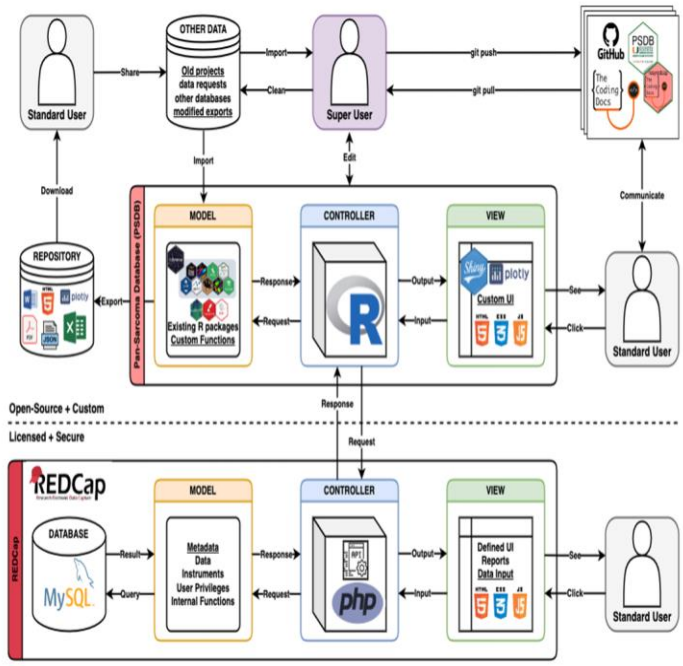
and store data and metadata, and uses open-source coding in R to transform, merge, explore, and analyze data. This process can be modified and customized to fit other institutions. We aim to share our data structure and process to establish basic standards in hopes of increased data sharing and collaborative research with institutions that treat and study patients with sarcoma.

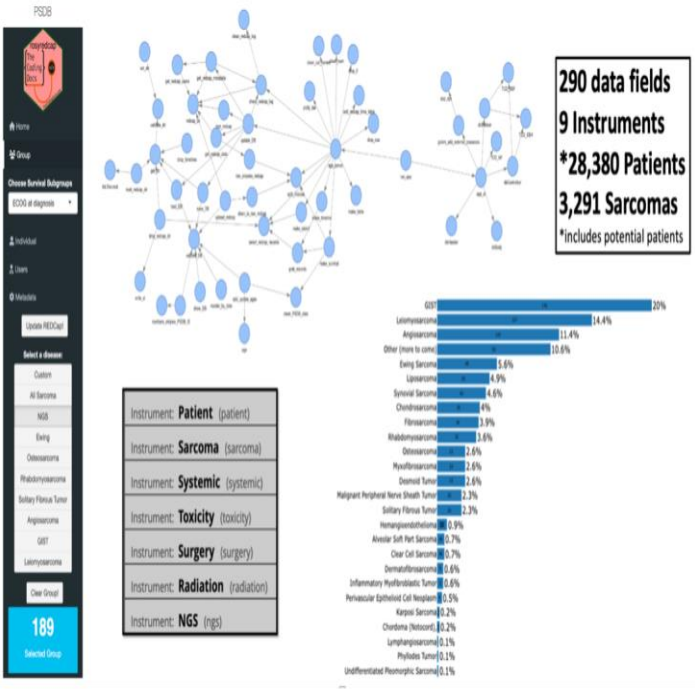
Methods: PSDB implemented a Model-View-Controller (MVC) framework, a software design pattern used by many modern-day applications. In this framework, "model" is the database, including the structure and metadata, "view" is the user interface software, and "controller" is the coding language used to handle user input, update the model, and interact with the viewer. PSDB uses REDCap, shiny, and R, respectively. REDCap, a free software, provides a centralized location for data storage, strict user privileges, logged data modifications, and has a robust application program interface (API). Shiny is an open-source R package that provides a way to create a custom user interface to explore, transform, and visualize data. Lastly, the R programming language for statistical computing provides extensive packages for data manipulation, visualization, and analysis. R is also used to create a custom package of functions for sophisticated data quality checks and custom data uploads using the REDCap API. We integrated other concepts in our design such as normalization and standards from Clinical Data Interchange Standards Consortium (CDISC). For example, partial dates are saved as partial and imputed as needed during analysis. As another example, data that is singular to a patient such as the medical record number, birth date, demographics, and survival status are stored at the highest level and data that can repeat, such as a sarcoma diagnosis or treatments are stored at another level, similar to relational databases. R code is mapped to our “model” and its associated transformation process to produce updating tables, time-to-endpoint analyses, and other modifiable outputs.

Results: The PSDB successfully addresses the unique data challenges faced in sarcoma research since it is comprised of a heterogenous set of mesenchymal cancers. The use of the MVC framework with REDCap, shiny, and R, enabled the creation of an application that is secure, user-friendly, and adaptable. The database is currently comprised of 290 data fields to capture demographics, survival data, sarcoma data, and associated treatment data. We continue to integrate multiple data sources, such as an internal cancer database, pathology records, and prior research data. We captured data from 28,380 candidate sarcoma patients from 2010 to present, including 3,291 confirmed sarcoma cases, with 751 cases reviewed to date. By capturing key data and organizing an open-source code base around it, we automated time-to-endpoint analyses that are updated as the data changes. Amongst our confirmed cases we have a cohort that is 53.3% female and 32.1% Hispanic. Amongst the cases reviewed so far we have a diverse group of histopathologies: GIST (176), Leiomyosarcoma (127), Angiosarcoma (100), Unspecified/Other (93), Ewing Sarcoma (49), Liposarcoma (43), Synovial Sarcoma (40), Chondrosarcoma (35), Fibrosarcoma (34), Rhabdomyosarcoma (32), Desmoid Tumor (23), Myxofibrosarcoma (23), Osteosarcoma (23), Malignant Peripheral Nerve Sheath Tumor (20), Solitary Fibrous Tumor (20), Hemangioendothelioma (8), Alveolar Soft Part Sarcoma (6), Clear Cell Sarcoma (6), Dermatofibrosarcoma (5), Inflammatory Myofibroblastic Tumor (5), Perivascular Epithelioid Cell Neoplasm (4), Chordoma (Notocord), (2), Karposi Sarcoma (2), Lymphangiosarcoma (1), Phyllodes Tumor (1), Undifferentiated Pleomorphic Sarcoma (1). We have several active projects working to grow our database. Currently, 30.6% of our cases are metastatic at diagnosis; 57.7% are High grade, 28.9% are Intermediate grade, and 8.9% low grade; the median age at diagnosis is 55; 93.5% of reviewed cases received systemic therapy and 73.1% received radiation.

Conclusion: The Pan-Sarcoma Database (PSDB) presented in this study represents an innovative approach to address the data management needs of a clinical research team focusing on sarcoma oncology. By employing the MVC software design pattern and leveraging

REDCap, shiny, and R, the PSDB provides a secure, flexible, version-controlled, reusable, and accessible clinical database application. The successful implementation of the PSDB facilitated efficient data collection, integration, and management, supporting collaborative research efforts and potentially enabling future applications of AI. This project emphasizes the importance of employing appropriate tools for effective data management and encourages similar initiatives to enhance collaboration and benefit both research teams and the patients they study.





(P 226) SIGNATERA TESTING TO IDENTIFY MINIMAL RESIDUAL DISEASE IN HIGH GRADE SARCOMA

Brandon E. Rose, MD, MPH[1]; Priscila Barreto coelho, MD[2]; Steven Bialick, DO[3]; Osvaldo Nunez, Jr.[4]; Solange Sierra, APRN[5]; Ty Subhawong, MD[2]; Francesco Alessandrino, MD[6]; Aditi Dhir, MD[4]; Emily Jonczak, MD[7]; Andrew Rosenberg, MD[6]; Gina Z. D'Amato, MD[8]; Jonathan Trent, MD[7], (1) University of Miami and Jackson Health Systems, Miami Beach, FL, (2) University of Miami, Miami, FL, (3) Sylvester Comprehensive Cancer Center, University of Miami, Deerfield Beach, FL, (4) University of Miami Miller School of Medicine, Miami, FL, (5) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, (6) Sylvester Comprehensive Cancer Center, MIAMI, FL, (7) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL, (8) Sylvester Comprehensive Cancer Center, Miami, FL

Objective: Soft tissue sarcomas are a rare group of tumors accounting for more than 150 different subtypes. Slightly more than half of sarcomas are diagnosed at early stage with 5-year survival rate of 81%. Approximately 20% are found at the locally advanced stage (stage 3) with 5-year survival rates ranging from 25-58%. Tumors presenting large (>5 cm), intermediate to high grade, and more aggressive subtypes have high early relapse rates necessitating active surveillance. Current surveillance involves physical exam and radiologic imaging which can be inconclusive and necessitate additional imaging and radiation exposure. Non-invasive biomarkers are needed to better monitor disease recurrence and responses to treatment. Signatera is a highly sensitive custom-built circulating tumor DNA (ctDNA) test for treatment monitoring and molecular residual disease (MRD) assessment in patients previously diagnosed with cancer, designed for each patient to help identify relapse earlier than standard surveillance. Our institution utilized this technology on our sarcoma patients and here we report on how the clinical courses of our patients were altered based on Signatera test results.

Methods: We performed standard of care for 32 high-grade/high risk soft tissue sarcoma patients who were treated locally and monitored with close surveillance consisting of physical examination and frequent imaging. In between scans patients received Signatera ctDNA testing. Radiology interpretation was performed by radiologists who specialize in sarcoma. Additional methods will be presented at CTOS.

Results: Our cohort consisted of 21 male (65.6%) and 11 female (52.4%) patients. Of the 32 sarcoma patients being followed for MRD using Signatera testing, we had 86 surveillance tests, of which 49 had associated imaging. There was a match in Signatera result and subsequent image result in 46 out of 49 cases (94.0%). We identified 3 patients who had evidence of MRD prior to a scheduled scan. Updated results with more patients and outcomes will be reported at CTOS and will include imaging.

Conclusion: Utilizing ctDNA technologies such as Signatera can potentially significantly improve patient outcomes. In this pilot study, our findings were similar to previously reported data by the Standford group. With short interval follow up, there were 3 patients that had earlier detection of their recurrent sarcoma than seen on imaging which altered their disease course. In addition, our preliminary data showed that all of negative ctDNA correlated with imaging results. Updated results with more patients and outcomes will be reported in the updated abstract. Further studies are warranted especially to investigate if early detection and help treating physicians make decisions about the controversial use of adjuvant chemotherapy for

high-risk soft tissue sarcomas.

(P 227) DE NOVO NATURAL LANGUAGE PROCESSING (NLP) ALGORITHM ACCURATELY IDENTIFIES MYXOFIBROSARCOMA DIRECTLY FROM PATHOLOGY REPORTS

Sarah E. Lindsay, MD[1]; Cecelia Madison, MS[2]; Duncan Ramsey, MD, MS[1]; Yee-Cheen Doung, MD[1]; Kenneth R. Gundle, MD[2], (1) Oregon Health & Science University, Portland, OR, (2) Portland VA Medical Center, Portland, OR

Objective: The rarity and heterogeneity of soft tissue sarcoma (STS) make conducting appropriately powered studies challenging. Large-scale databases have the potential to be useful in investigating soft tissue sarcoma clinical outcomes. Many databases rely on ontologies, such as International Classification of Diseases (ICD) codes. However, ICD codes often do not accurately reflect sarcoma diagnoses, and this results in an underrepresentation of sarcoma in large registries and databases (1). Natural language processing (NLP) is a branch of computer science that has been applied to clinical documentation for the purposes of data extraction. It has the potential to be applied in the setting of STS to clinical documents like pathology reports to identify STS independent of ICD codes. The national VA database provides a unique opportunity for STS investigation, due to the availability of all clinical results and pathology reports. The purpose of this study was to train and test an NLP algorithm to analyze pathology reports to identify patients with myxofibrosarcoma. Our hypothesis was that utilizing NLP to analyze pathology reports would identify patients who lack International Classification of Diseases (ICD) codes for myxofibrosarcoma.

Methods: Institutional Review Board approval was obtained for this retrospective study. Through the national VA corporate data warehouse, all surgical pathology notes were identified from 2003 through 2022. Using the word search functionality, these reports were retrospectively searched for the term “myxofibrosarcoma.” The resulting pathology reports were then reviewed manually to yield a final cohort. ICD diagnosis codes were abstracted. An NLP algorithm was iteratively refined and tested using confounders, negation, and emphasis terms. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated for the NLP-generated cohort, and these values were compared to those of the manually reviewed gold-standard cohort.

Results: In searching 10.7 million pathology reports, we identified 388 patients with pathology reports that contained the term “myxofibrosarcoma.” Manual review confirmed that 302 patients had true myxofibrosarcomas (22% error rate). Sarcoma ICD codes were absent for 17% of these patients. Through iterative testing and training, the final NLP algorithm improved accuracy in identifying myxofibrosarcoma cases compared to utilizing ICD codes or basic word searches ($p < 0.001$). The algorithm flags 4% of reports for manual review. For the remaining 96% of pathology reports, our algorithm has a sensitivity of 99.7%, a specificity of 93.0%, a positive predictive value of 98.4%, and a negative predictive value of 98.5% for classifying reports as myxofibrosarcoma or an alternative diagnosis.

Conclusion: NLP algorithms can identify patients with myxofibrosarcoma from pathology reports with high accuracy, which is an improvement over ICD-based cohort creation. This algorithm will be freely released to facilitate external validation and improvement through testing in other cohorts. Our next step is to apply this algorithm to additional soft tissue sarcoma

diagnoses as we aim to build a comprehensive database to better study sarcoma incidence and outcomes. Level of Evidence: Diagnostic Study, Level II Works Cited: 1. Lyu, H. G. et al. Assessment of the Accuracy of Disease Coding Among Patients Diagnosed With Sarcoma. JAMA Oncol. 4, 1293–1295 (2018).

(P 228) WHAT IS THE SIGNIFICANCE OF INDETERMINATE PULMONARY NODULES IN HIGH GRADE SOFT TISSUE SARCOMA? A RETROSPECTIVE COHORT STUDY

Marcus J. Brookes, MBBS, MRes, MRCS[1]; Corey D. Chan, MBBS, MRCS, MRes, PGCert[2]; Timothy Crowley, MBBS, FRCS[1]; Maniram Ragbir, MBBS, FRCS[1]; Thomas Beckingsale, MBBS, MSc, FRCS[1]; Kanishka M. Ghosh, MBBS, MD, FRCS[1]; Kenneth S. Rankin, MB ChB, MD, FRCS[1], (1) The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, England, United Kingdom, (2) Newcastle University, Newcastle upon Tyne, England, United Kingdom

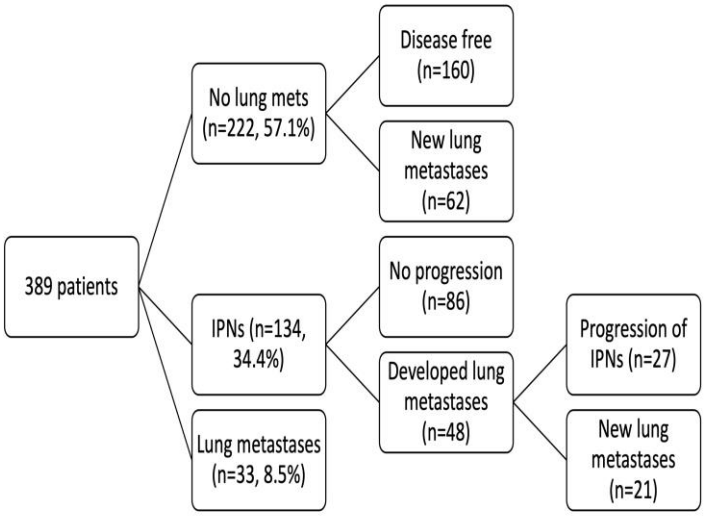
Objective: Sarcomas are rare, aggressive cancers which frequently metastasise to the lungs. Following diagnosis, patients typically undergo staging by means of a CT scan of their chest. Whilst the scan often identifies no lung nodules or frank metastases, indeterminate pulmonary nodules (IPNs) are often reported. Delineating between benign and malignant lung nodules is important with regards to treatment decision making, with complete resection of metastatic disease important for survival in sarcoma. Whilst the presence of IPNs has been shown to be associated with decreased overall survival (OS) in osteosarcoma, the significance of IPNs in high grade soft tissue sarcoma (STS) is unclear. This study aims to ascertain the clinical relevance of IPNs in patients with high grade STS, their effect on survival and factors associated with their progression to metastatic disease.

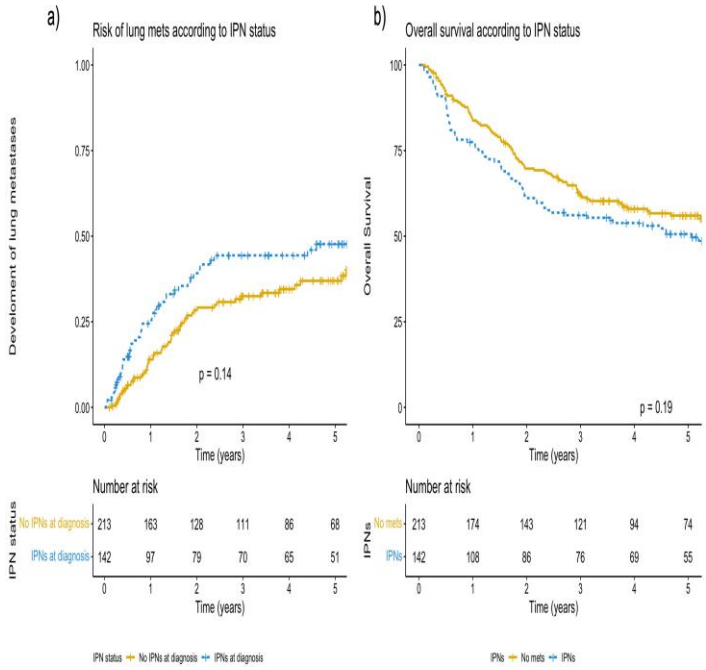
Methods: All patients treated at the North of England Bone and Soft Tissue Tumour Service for high grade STS between 01/01/2010 and 01/05/2020 were identified from a prospectively maintained database. Patient were excluded if they had low grade sarcomas, intracranial, retroperitoneal or visceral locations, of if we had no access to their staging scans. IPNs were classified as non-calcified lung nodules < 10mm in diameter. CT staging scans and reports were reviewed, as well as any subsequent imaging, and survival data collected from patient records. In patients with IPNs at diagnosis developing lung metastases, it was noted whether this was progression of the IPNs or the development of new lesions. Statistical analysis was conducted in SPSS and R studio.

Results: 389 patients were identified as suitable and included in the study. Patients had a median age of 61.9 years old and there was a male predominance of 62.4%. On CT staging scans, 57.1% of patients had no signs of lung metastases, 34.4% of patients had IPNs and 8.5% had frank lung metastases (Figure 1). There was a trend towards increased risk of developing lung metastases and poorer OS in patients with IPNs, although neither reached significance (p=0.14 and p=0.19 respectively) (Figure 2). Of those presenting with IPNs, 20.1% progressed to metastases; they were more likely to progress if IPNs were 5mm or greater in diameter (p=0.006), multiple IPNs (p=0.013) or bilateral IPNs (p=0.022), as well as in patients with primaries 5cm or larger (p=0.014), grade 3 primaries (p=0.009) or primaries arising deep to the fascia (p=0.041) (Table 1). The median time to progression of IPNs was 143 days; 18.5% of IPNs that progressed did so after 12 months however. Patients with undifferentiated

pleomorphic sarcoma (n=100) and myxofibrosarcoma (n=100) were analysed independently; IPNs at diagnosis were not associated with an increased likelihood of developing lung metastases or with decreased OS in either subtype.

Conclusion: It is crucial to consider the primary tumour as well the IPNs when considering the risk of progression. Surveillance CT scans should be carried out within 6 months and repeated at 12 months, although improved imaging technologies are necessary to improve differentiation between benign and malignant nodules. The association between IPNs and survival in patients with high grade STS remains unclear and requires further study. Further study is also required to ascertain the influence of histological subtype on the risk of IPN progression.





Progression of IPNs				
	Univariate		Multivariate	
	HR	p value	HR	p value
IPN number ≥1	2.76	0.013*	2.29	0.087
IPN size ≥5mm	2.96	0.006**	2.37	0.030*
Bilateral IPNs	2.43	0.022*	1.66	0.282
Primary size ≥5cm	4.55	0.014*	2.58	0.160
Primary grade	6.79	0.009**	6.07	0.015*
Primary depth	2.46	0.041*	1.71	0.280

(P 229) ON THE VARIABILITY IN THE ANALYSIS OF APPARENT DIFFUSION COEFFICIENT ESTIMATES FOR MULTICENTER DIFFUSION MRI DATA OF RHABDOMYOSARCOMA PATIENTS

Giulia De Donno, MSc[1]; Cyrano Chatziantoniou[2]; Alexander Leemans, Dr. Dr.h.c.mult.[2]; Reineke A. Schoot, MD PhD[3]; Rick R. Van Rijn, Prof. MD. PhD.[4]; Johannes H. M Merks, Prof. dr.[5]; Alberto De Luca, Dr.[1]; Roelof van Ewijk, MD[6], (1) Image Sciences Institute, UMC Utrecht, Utrecht, Utrecht, Netherlands, (2) Image Sciences Institute, UMC utrecht, Utrecht, Utrecht, Netherlands, (3) Princess Máxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands, (4) Department of Radiology and Nuclear Medicine, Amsterdam UMC – Emma Children's Hospital, University of Amsterdam, Amsterdam, Noord-Holland, Netherlands, (5) Princess Máxima Center for Pediatric Oncology and Division of Imaging and Oncology, University Medical Center Utrecht, University of Utrecht, Utrecht, Utrecht, Netherlands, (6) Princess Maxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands

Objective: Pediatric rhabdomyosarcoma (RMS) is a common and aggressive type of soft tissue sarcoma that requires multimodal therapy and can be found in different parts of the body. In this study, retrospective data from a multicenter longitudinal study (EpSSG RMS-2005 study) is used to evaluate the diagnostic potential of diffusion MRI in RMS. As multicenter studies become increasingly more common in clinical trials, it is crucial to acknowledge that apparent diffusion coefficient (ADC) values may be influenced by various factors such as acquisition parameters, scanner manufacturers, pre and post processing, type of segmentation and pipeline used to create the ADC maps. Our goal is to compare ADC map generated by different scanners to ADC maps computed by a standardized in-house pipeline from diffusion weighted images (DWI), to determine whether these factors might contribute to a potential decrease in sensitivity in measurement across multiple centers.

Methods: In our study we included 144 sets of images from 67 pediatric patients. Each set has: an ADC map automatically generated from the scanner, an ADC map computed by our in-house pipeline using existing DWI data, and a binary mask that delineates the non-necrotic regions of the tumor. In both ADC maps, outliers from noise were removed using the 1.5 interquartile range rule. Among the patients, longitudinal data was available for 36 individuals, consisting of one scan at diagnosis and another after induction chemotherapy (follow up). The study aims to compare ADC values obtained from the scanner with those calculated from DWI within the tumor across all images (I), and with focus on longitudinal data (II), using relative percent difference between images and difference of ADC values for each voxel within the mask. Paired t-test was used for statistical analysis.

Results: (I) In each of the 144 sets of images (few examples are shown in Fig. 1), the average voxel-wise relative percentile difference between ADC from scanner and DWI within the tumor is 5.94 ± 27.94 % (range -8.30, +200.00 %, median value 0.89%). This is linked to a median voxel-wise difference in ADC values (ADC from DWI – ADC from scanner) of 0.04 ± 0.18 ·10-3 mm2/s (range -0.15, +1.58 ·10-3 mm2/s, median value 0.01·10-3 mm2/s). No statistical difference in median relative difference has been found for different manufacturers (GE, Philips, Siemens).

(II) Fig. 2 depicts an example of scan at diagnosis and follow up, and relative histograms of ADC from scanner and DWI. As shown in Fig. 3, the median ADC values from the scanner at diagnosis are 1.16 ± 0.32 ·10-3 mm2/s, and at follow-up are 1.51 ± 0.36 ·10-3 mm2/s. On the other hand, median ADC values from DWI at diagnosis are 1.18 ± 0.31 ·10-3 mm2/s and at follow-up are 1.55 ± 0.35 ·10-3 mm2/s. Taking into consideration the median values of the

segmented areas, statistically significant differences have been found between diagnosis and follow up, but not between ADC from the scanner and DWI at the same time point.

Conclusion: Our results show that the relative difference within the tumor between ADC from scanner and DWI can vary substantially. As shown in Fig. 1, difference in ADC values might be caused by the different pipeline used to generate the image, as well as preprocessing steps on the ADC map from scanner. Even if differences in median ADC values from scanner and DWI do not have a statistical difference in this cohort, we see how ADC values at diagnosis have consistently lower values compared to the ones at follow up (Fig. 2, Fig. 3). It is important to be aware of these differences, as they can potentially create another layer of variability in a multicenter study. Moreover, ADC maps generated from different scanners can undergo different pre and postprocessing steps, of which the user might not be aware of. A standardized pipeline for generating ADC maps across all images in the dataset would mitigate unwanted variability and enhance reliability and comparability across the dataset.

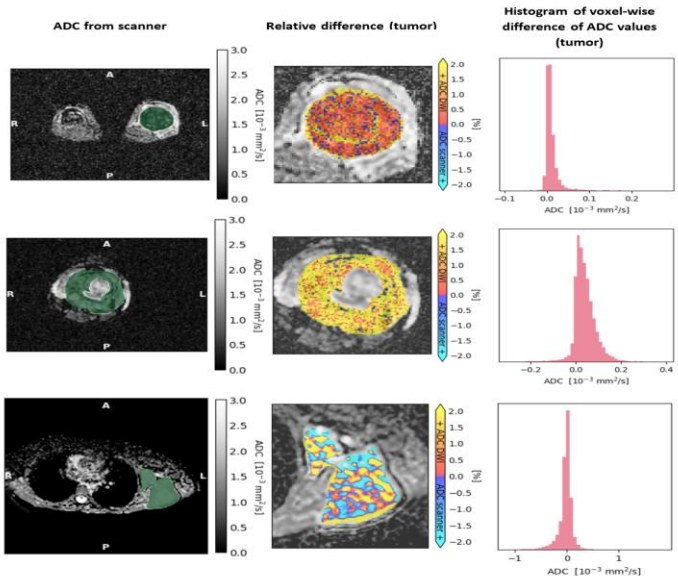


Figure 1. *In the first column, a representation of the ADC map generated from the scanner and relative segmentation of the non-necrotic areas of the tumor (green). In the second column, a close up of the segmented area, with the relative percentage difference shown as a color map: red-green for areas in which the ADC from the DWI has higher values, in blue and light blue areas in which the ADC from the scanner is higher. On the last column, an histogram of the voxel-wise difference between ADC from the DWI and ADC from the scanner. In the first two rows, we have an example of longitudinal data, on the third an example of patterns of relative difference, possibly created from pre-processing steps instead of underlying anatomy.*

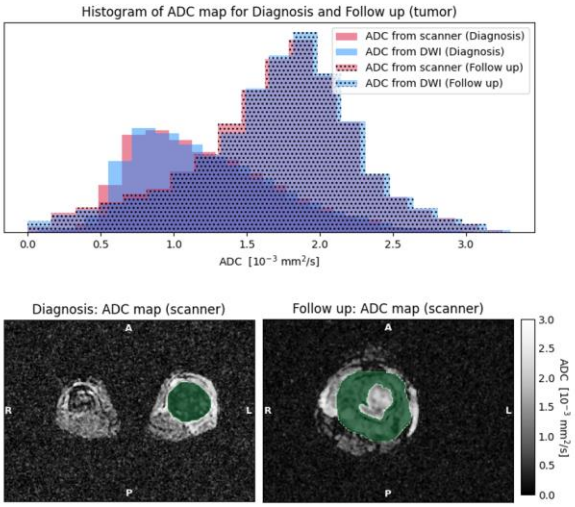
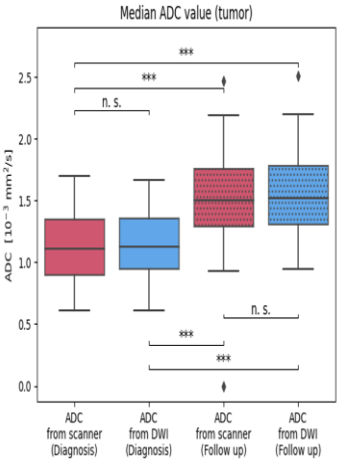


Figure 2. *An example of longitudinal data. In the first row, the histogram of the ADC within the non-necrotic areas of the tumor are represented. Consistently with the rest of the study, ADC values from follow up images show higher median ADC values compared to the ones from diagnosis. In the second row, longitudinal ADC maps and relative tumor segmentation (green). In this example, the field of view is different, and so is the amount of values within the segmentation, independently from anatomical changes. In case the ADC map is generated directly from the scanner, we have limited information regarding pre and post processing steps used to generate the map, as well as the method used to calculate the ADC values. Moreover, these longitudinal images might be scanned with different acquisition parameters and from different scanners, potentially adding variability to the measurement.*



	ADC scanner Diagnosis	ADC DWI Diagnosis	ADC scanner Follow Up
ADC DWI Diagnosis	7.81e-02		
ADC scanner Follow Up	1.78e-05	3.66e-05	
ADC DWI Follow Up	8.09e-08	2.02e-07	1.18 e-1

Figure 3. *Comparison of median values in ADC maps from scanner and DWI at diagnosis and follow up for the 36 sets of longitudinal images. On the left, the boxplots of the median values has indicated the statistical significance calculated from paired t-tests (p-values > 0.05 = not significant (n.s.), p-values < 0.05 are indicated with 1 asterisk (*), p-values < 0.01 with (**), p-values < 0.001 with (***)). On the right, a table indicating the exact p-values from the pairwise paired t-tests. Statistically significant differences in ADC values between diagnosis and follow up indicate that this measure has potential to be used as biomarker for treatment response.*

(P 230) SARCDBASE: A TOOL FOR DETECTION OF GENETIC ALTERATIONS IN SARCOMA

Valeria Difilippo, Master[1]; Karim H. Saba, PhD[1]; Karin Wallander, MD, PhD[2]; Michaela Nathrath, N/A, MD[3]; Daniel Baumhoer, MD[4]; Felix Haglund de Flon, MD, Ph.D[5]; Karolin Hansén Nord, PhD[1], (1) Lund University, Lund, Skane Lan, Sweden, (2) Karolinska Institutet, Stockholm, Stockholms Lan, Sweden, (3) Klinikum Kassel, Kassel, Hessen, Germany, (4) University Hospital Basel, Basel, Basel-Stadt, Switzerland, (5) Karolinska University Hospital, Stockholm, Stockholms Lan, Sweden

Objective: Sarcomas are a diverse group of malignant tumors arising in the bone and soft tissues. For an increasing number of subtypes, there are already pathognomonic genetic alterations described. For some sarcomas, such alterations remain to be identified. The latter group primarily contains sarcomas with a massive amount of genomic copy number and structural alterations. This extreme number of alterations makes it difficult to discriminate those of biological importance from background noise. We have developed SarcDBase, a database-building method that matches genetic variants detected in high-resolution genomic and transcriptomic data from tumor biopsies with information on established biomarkers, eliminating the need for prior knowledge and manual screening of data.

Methods: The tool is divided into three main sections. Data analysis: DNA and RNA sequencing data are used to detect (i) structural variants, (ii) single nucleotide variants and insertions/deletions, (iii) genome-wide DNA copy number changes, and (iv) gene fusions. The latter step includes the tool NAFuse (https://github.com/ValeriaDifilippo/NAFuse), which is an integrated methodology that combines and compares the output of an RNA gene fusion detector with that of the BAM-file generated by whole genome DNA sequencing. In this way, gene fusions detected at both the RNA and DNA levels can be extracted. SarcDBase execution: a series of codes that use external sources, such as the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, the WHO classification of tumors (Soft Tissue and Bone Tumours), and other literature sources, to detect previously reported genetic alterations. SarcDBase also detects novel alterations of likely biological significance, such as new combinations of partner genes in gene fusions. SarcDBase visualization: an app in dash that helps the user to visualize and interpret the data with a graphical interphase and to have a dynamic visualization.

Results: The app in dash allows a dynamic navigation through the data and the user can navigate through multiple pages depending on the data available. Here, the performance of SarcDBase was tested on a diverse cohort of deep-sequenced sarcomas (n = 70). Several mutations of likely biological significance were identified. Among these, we found mutations that confirmed the diagnosis in some cases, such as a NAB2::STAT6 gene fusion (solitary fibrous tumor), an H3F3A p.G35W (giant cell tumor of bone), an SS18::SSX1 gene fusion (synovial sarcoma), a JAZF1::PHF1 gene fusion (endometrial stromal sarcoma) and a non-frameshift deletion of KIT (gastrointestinal stromal tumor). SarcDBase was also able to identify new genetic alterations of likely biological significance, such as novel combinations of partner genes in gene fusions.

Conclusion: SarcDBase is a useful tool that integrates genomic and transcriptomic information to extract previously reported and novel genetic alterations. It can be used on any deep sequencing data from tumor tissue and the graphical visualization part helps the user to evaluate the findings.

(P 231) MINIMALLY INTERACTIVE SEGMENTATION OF SOFT-TISSUE TUMORS ON MRI AND CT USING DEEP LEARNING

Douwe J. Spaanderman, MS[1]; Martijn P A Starmans, PhD[1]; Gonnie van Erp, MS[1]; David Hanff, MD[1]; Judith Sluijter, MS[1]; Anne-Rose W. Schut, MD[1]; Geert Leenders, MD, PhD[1]; Cornelis Verhoef, MD, PhD[1]; Wiro J. Niessen, N/A, PhD[2]; Jacob J. Visser, MD, PhD[1]; Dirk J. Grünhagen, MD, PhD[1]; Stefan Klein, PhD[1], (1) Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands, (2) University of Groningen, Groningen, Groningen, Netherlands

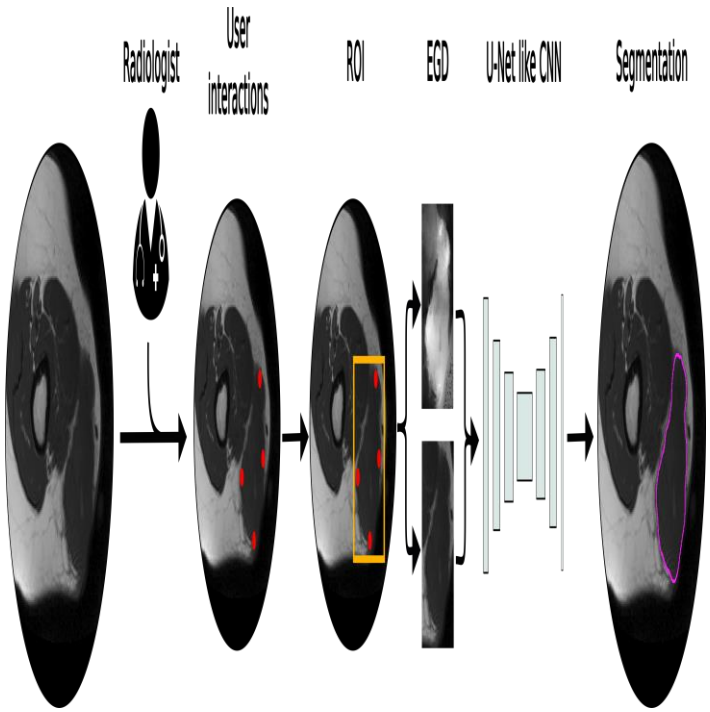
Objective: Segmentations have many applications in soft-tissue tumor (STT) healthcare, as they can be used for clinical measurements, e.g., to evaluate therapy response (i.e., RECIST), to target radiotherapy, and are often required to extract quantitative imaging biomarkers (radiomics). Manual segmentation, although accurate, is time-consuming and observer-dependent, and thus not feasible in a radiologist's clinical workflow. Automatic methods, on the other hand, may have limited accuracy due to the heterogeneous nature of STTs. In this study, we present a minimally interactive segmentation method, in which the radiologist guides a deep-learning network to generate segmentations.

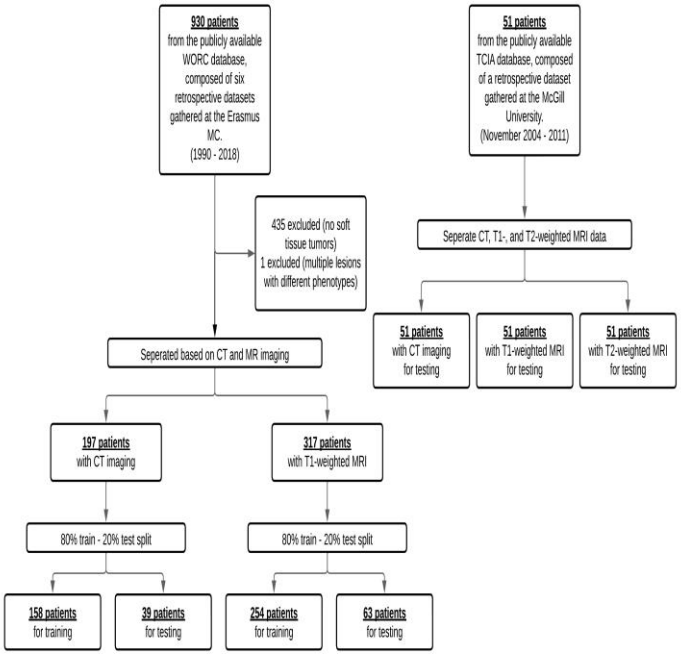
Methods: Our interactive method requires the user to manually mark six points near the extreme boundaries of the tumor, i.e., two extreme points for all three directions, in the image (Fig 1). The points are used to extract a region of interest (ROI) around the tumor and are transformed into an exponentialized geodesic distance map (EGD), which indicates the location of the tumor by calculating the distance in contrast between the interactions and surrounding tissue. Together the ROI and EGD serve as input for a Convolutional Neural Network (CNN). We evaluated our interactive segmentation method in the public WORC database, which included 514 patients with 11 STT phenotypes on either CT or T1-weighted MRI (Fig 2). We trained separate networks for MRI and CT and split the data in 80% for training and 20% for test. Next, we validated the interactive method in another public dataset from McGill University, referred to as the TCIA dataset, which included 51 patients with 7 STT phenotypes on CT, T1- and T2- weighted MRI. Moreover, 5 STT phenotypes included in the TCIA dataset were not presented in the 11 STT phenotypes of the WORC database. Therefore, the TCIA dataset was also used to assess the performance for STT phenotypes not seen during training of the methods. We compared the interactive segmentation method to automated segmentation using the nnU-Net framework. Performance was measured using the Dice Similarity Coefficient (DSC). The DSCs of the segmentation methods were compared with paired two-sided t-tests. Additionally, we evaluated the clinical utility of the segmentation methods by calculating correlation scores between volumetric and diameter measurements compared to ground truth segmentation.

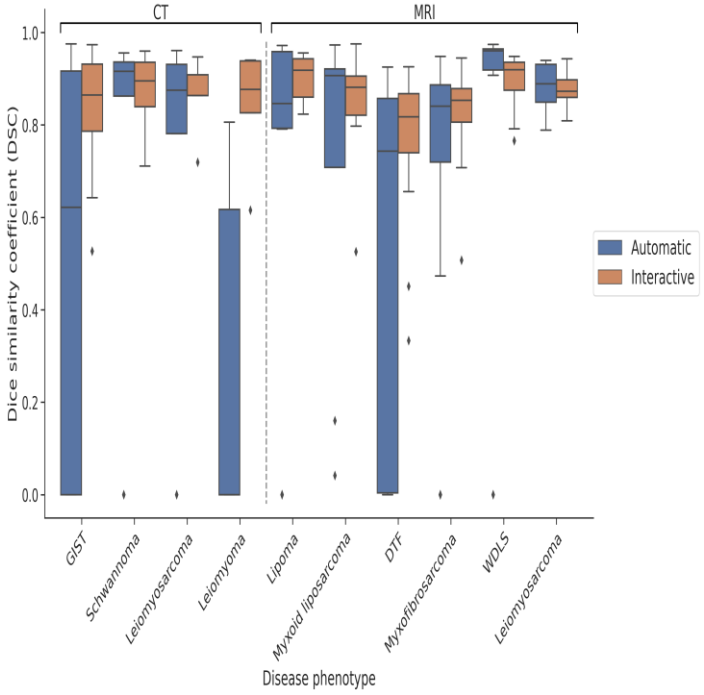
Results: For interactive segmentation, we obtained a mean (±standard deviation) test DSC of 0.85±0.11 for CT and 0.84±0.12 for T1-weighted MRI on the WORC test dataset (Fig 3). The automatic segmentation method obtained significantly worse results (CT: 0.52 ± 0.43 ($p< 0.001$), T1-weightedMRI: 0.71 ± 0.35 ($p=0.007$)). Similarly, volume and diameter calculations based on the interactive segmentation had a better agreement (volume: $r=0.96$, diameter: $r=0.99$) than on automatic segmentation (volume: $r=0.76$, diameter: $r=0.62$) when compared to the ground truth segmentation. Validation of the interactive segmentation method on TCIA data showed similar performances in terms of DSC on all sequences (CT: 0.81 ± 0.08, T1: 0.84 ± 0.09, T2: 0.88 ± 0.08), while automatic segmentation performed worse (CT: 0.38 ± 0.341, T1: 0.81 ± 0.234, T2: 0.78 ± 0.216). Here, interactive segmentation significantly outperformed automatic segmentation on CT and T2-weighted MRI, while providing similar results on T1-

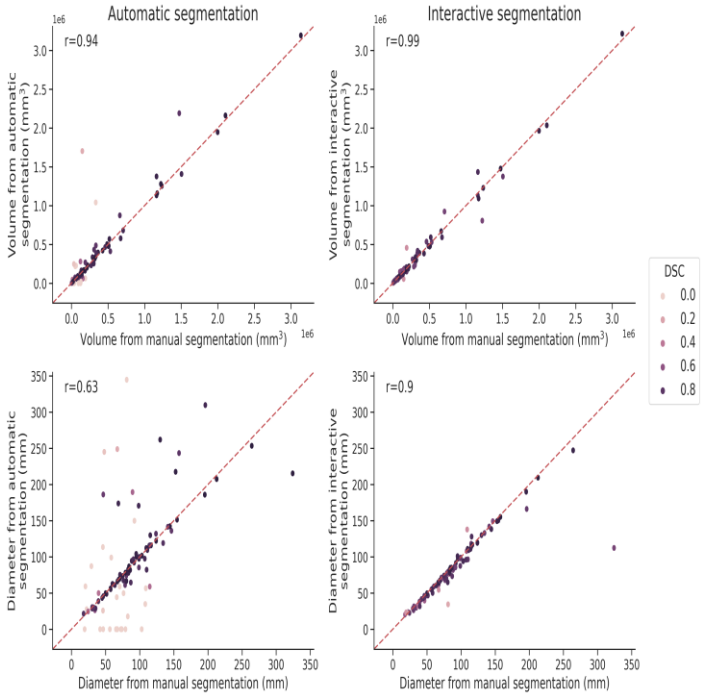
weighted MRI (CT: p< 0.001, T1-weighted MRI: p=0.14, T2-weighted MRI: p=0.004).

Conclusion: Our minimally interactive model generated semi-automatic segmentations with higher DSC than automatic segmentation for various STT types on T1-weighted MRI and CT. Additionally, we showed that the interactive segmentation method can be used to accurately perform volume and diameter measurements. Furthermore, minimally interactive segmentation can be used to detect unseen tumor types and STT on modalities not seen during the training of the model. Based on these results, we are currently investigating the use of interactive segmentation in the clinical workflow.




930 patients
from the publicly available WORC database, composed of six retrospective datasets gathered at the Erasmus MC.
(1990 - 2018)
51 patients
from the publicly available TCIA database, composed of a retrospective dataset gathered at the McGill University.
(November 2004 - 2011)
435 excluded (no soft tissue tumors)
1 excluded (multiple lesions with different phenotypes)
Seperate CT, T1-, and T2-weighted MRI data
51 patients
with CT imaging for testing
51 patients
with T1-weighted MRI for testing
51 patients
with T2-weighted MRI for testing
Seperated based on CT and MR imaging
197 patients
with CT imaging
317 patients
with T1-weighted MRI
80% train - 20% test split
80% train - 20% test split
158 patients
for training
39 patients
for testing
254 patients
for training
63 patients
for testing


CT
MRI
Dice similarity coefficient (DSC)
1.0
0.8
0.6
0.4
0.2
0.0
Automatic
Interactive
GIST
Schwannoma
Leiomyosarcoma
Leiomyoma
Lipoma
Myxoid liposarcoma
DTF
Myxofibrosarcoma
WDLS
Leiomyosarcoma
Disease phenotype



(P 232) NOVEL MRI SCORING SYSTEM TO ASSESS OSSEOUS MALIGNANCY IN SOFT TISSUE SARCOMA PATIENTS FOLLOWING RADIOTHERAPY

Gayathri Vijayakumar, BS[1]; Conor Jones, MD[2]; Stephen Supple, MD[2]; Austin Yu, BS[3]; Alan T. Blank, MD, MS[2]; John Meyer, DO[2], (1) Rush University Medical Center, Edison, NJ, (2) Rush University Medical Center, Chicago, IL, (3) Rush University Medical Center, Alhambra, CA

Objective: Radiation induced changes in bone such as radiation osteitis are commonly identified on magnetic resonance imaging (MRI) in patients who receive radiotherapy for soft tissue sarcoma (STS) management. This study proposes a novel MRI scoring system to assess osseous lesions and predict potential for malignancy based on MRI score in STS patients who received radiotherapy.

Methods: The MRI score consisted of 3 parameters: morphology, signal intensity, and progression. Interobserver reliability between MRI scores were analyzed with Cohen's kappa coefficient. Receiver operating curve (ROC) analysis was performed to determine a predictive MRI score for malignancy.

Results: 156 MRI's from 30 STS patients who received radiotherapy were retrospectively reviewed. Two (6.7%) patients developed regional osseous metastasis identified on MRI. The kappa coefficient of the scoring system was 0.785 demonstrating substantial interobserver agreement ($p < 0.001$). ROC analysis demonstrated that the optimal cut-off value for malignant lesion on MRI was 5.5 (area under the curve 0.998; $p < 0.001$).

Conclusion: This novel MRI scoring system recommends lesions with a score of six and above

to be biopsied to distinguish if malignancy is present. We believe this scoring system can be utilized by multidisciplinary care teams to guide clinical recommendations for patients with STS and MRI findings concerning for malignancy versus radiation induced changes.

Table 1. Supple Score-MRI Scoring System

	-1	0	1	2	3
Morphology	N/A	• Serpiginous or linear abnormality • Diffuse normal marrow signal	Diffuse/patchy signal abnormality on T2FS/STIR with ill-defined edges	Focal signal abnormality on T2FS/STIR with ill-defined edges	Focal nodular or ovoid signal abnormality on T2FS/STIR
Signal Intensity	N/A	Hyperintense T1 and Hypointense T2FS/STIR	Hyperintense on T2FS/STIR, Iso to hyperintense on T1	Hyperintense on T2FS/STIR, Hypointense on T1	N/A
Progression	Decreased signal abnormality	• N/A • Stable	New signal abnormality	Interval growth of the signal abnormality	N/A

Table 2. MRI Scoring System Recommendations

Score	Recommendation
0-3	Almost certainly benign. No specific follow up needed.
4-5	Probably benign, Imaging follow up is appropriate
6+	Suspicious for Metastasis, biopsy recommended

(P 233) MULTI-CENTER EXTERNAL VALIDATION OF AN AUTOMATED MODEL DIFFERENTIATING ALT FROM LIPOMA USING RADIOMICS AND DEEP-LEARNING BASED SEGMENTATION

Stefanie N. Hakkesteegt, MD[1]; Douwe J. Spaanderman, MS[1]; Anne-Rose W. Schut, MD[1]; David Hanff, MD[1]; Christina Messiou, MRCP, FRCP. MD[2]; Robin L. Jones, BSc, MB BS, MRCP, MD[3]; Andrew J. Hayes, MBBS FRCS, PhD[4]; Lorenzo Nardo, MD, PhD[5]; Yasser G. Abdelhafez, MD, PhD[5]; Ahmed W. Moawad, MD, PhD[6]; Khaled M. Elsayes, MD, PhD[6]; Sonia Lee, MD, PhD[7]; Thomas M. Link, MD, PhD[8]; Wiro J. Niessen, N/A, PhD[9]; Geert Leenders, MD, PhD[1]; Jacob J. Visser, MD, PhD[1]; Stefan Klein, PhD[1]; Dirk J. Grünhagen, MD, PhD[1]; Cornelis Verhoef, MD, PhD[1]; Martijn P A Starmans, PhD[1], (1) Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands, (2) The Royal Marsden Hospital, London, England, United Kingdom, (3) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (4) Royal Marsden Hospital, London, England, United Kingdom, (5) University of California Davis Health, Sacramento, CA, (6) University of Texas MD Anderson Cancer Center, Houston, TX, (7) University of California, Irvine, Irvine, CA, (8) University of California, San Francisco, San Francisco, CA, (9) University of Groningen, Groningen, Groningen, Netherlands

Objective: Lipomatous tumors are the most frequently encountered soft tissue tumors (STT), ranging from benign lipomas to malignant liposarcomas. Atypical lipomatous tumors (ALTs) are considered borderline tumors due to their locally aggressive growth patterns, tendency for recurrence, and risk for progression to dedifferentiated liposarcoma (DDLS) which can metastasize. As prognosis and treatment differs between ALTs and lipomas, it is vital to differentiate between these two phenotypes. Distinction is typically achieved through investigation of MDM2 amplification, which serves as a diagnostic marker for ALT, through Fluorescence In Situ Hybridization (FISH) testing on needle biopsy material. However, biopsies impose burdens on the patient and are susceptible to sampling errors.
Alternatively, Magnetic Resonance Imaging (MRI) gathered in routine patient care could provide insight in the differentiation of lipomas and ALT. Previously, we have proposed to differentiate between lipomas and ALTs with quantitative imaging features using machine learning (radiomics) to remove the need for invasive biopsies in the diagnostic process [1]. However, before introducing radiomics into clinical practice, external validation of our radiomics models is crucial to ascertain its generalizability and clinical applicability. Moreover, radiomics requires manual segmentation of the region of interest (ROI) to extract imaging features which requires expert knowledge, is time-consuming, and prone to interobserver variability, and thus unfeasible in the radiologist's clinical workflow. Automatic segmentation techniques using deep learning offers a potential solution by streamlining the process.
This study aims to externally validate our previously described radiomics model to differentiate between lipomas and ALTs on MRI in a large multi-center cohort [1]. Additionally, we extend our model with automatic and minimally interactive segmentation methods to increase the clinical feasibility [2].

Methods: Three different study cohorts were created. For training, we used the original training cohort previously described by Vos et al [1], which includes 48 Lipoma and 48 ALT patients from the Netherlands (Erasmus MC) and is publicly available [5]. This dataset includes manual segmentations made either by a medical MSc student or a medical PhD student under supervision of a musculoskeletal radiologist. For validation, two datasets were used. An external dataset was formed by data from the United States (UC Davis, MD Anderson, UCSF, and UC Irvine), and the United Kingdom (Royal Marsden). A prospective dataset contained

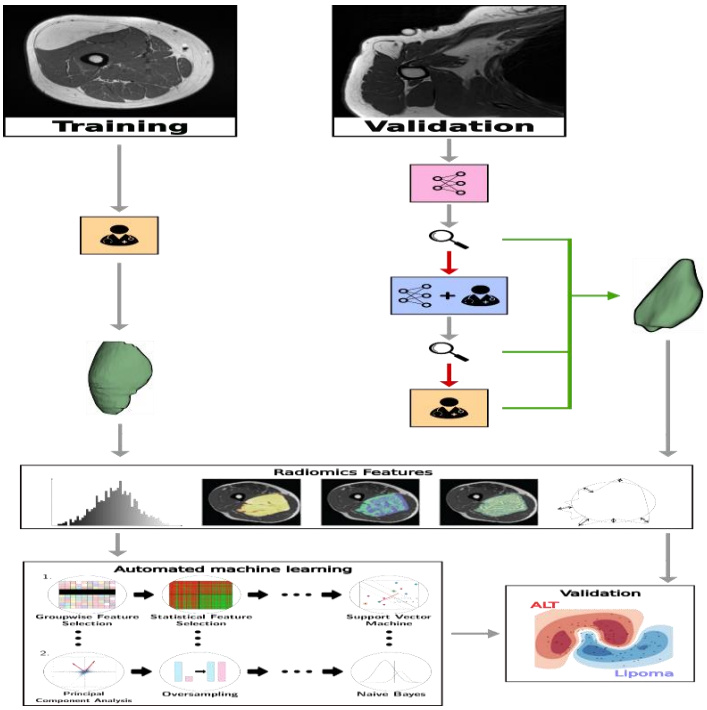
prospectively collected data from the Erasmus MC. For both cohorts, patient characteristics, tumor type, MDM2 amplification status, and T1-weighted MRI sequences were collected.
Our previously trained automatic segmentation method was used to automatically segment all lipoma and ALT lesions on the plain T1-weighted MRI scans of the validation cohorts [2]. Subsequently, segmentations were visually scored by a medical PhD student under the supervision of a musculoskeletal radiologist. Based on the percentage of volume overlap of the automatic segmentation with the tumor, segmentations were scored “Sufficient” (>75% overlap) or "Insufficient” (< 75% overlap) . When scored “Insufficient”, our interactive segmentation method was used instead [2]. Finally, in patients where the interactive segmentation also failed to provide an accurate segmentation, segmentations were manually adjusted (Figure 2).
To predict between MDM2 amplification status, we used the model as described by Vos et al. [1] based on the workflow for optimal radiomics classification (WORC) radiomics method [4]. WORC extracts imaging features from the tumor region such as intensity, shape, and texture features. Next, WORC searches, in a fully automated way, among a large pool of machine learning algorithms, including feature selection, dimensionality reduction, and classification algorithms, to find a combination which maximizes the prediction accuracy while maintaining robustness (Figure 1). Performance was evaluated using a 100 times random-split cross-validation on the training dataset, and for the validation datasets using 10000 times bootstrap resampling.

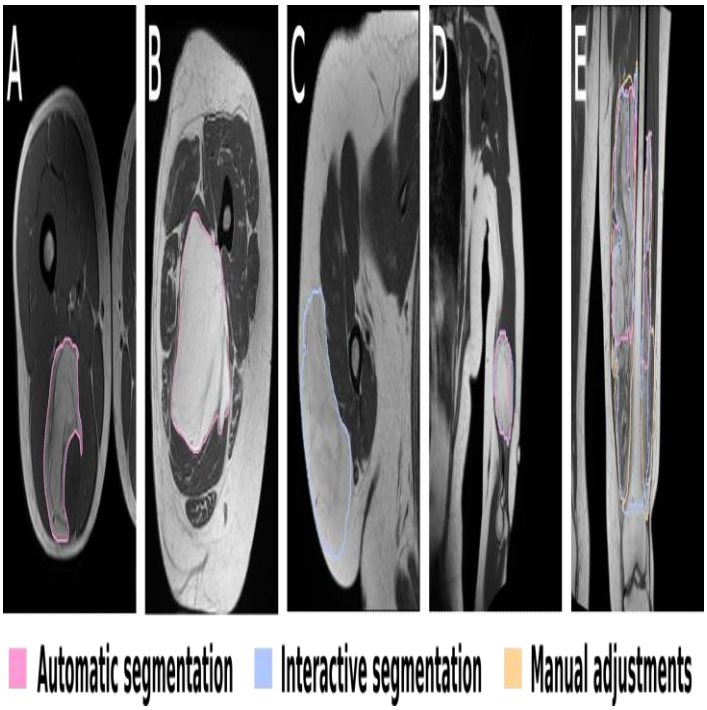
Results: The two validation datasets in total included 444 tumors (181 ALT, 263 lipoma), of which 86 (24 ALT, 62 lipoma) in the prospective validation cohort and 358 (157 ALT, 201 lipoma) in the external validation cohort. The mean age was 58 years and 50% of patients was male. In the two validation cohorts, 347 automatic segmentations were scored sufficient and 97 insufficient. The insufficient automatic segmentations were interactively segmented, resulting in 84 sufficient and 13 insufficient segmentations. These 13 insufficient interactive segmentations were manually adjusted.
The receiver operating characteristic curves of the model are illustrated in Figure 3; the metrics are reported in Table 1. The radiomics model based on T1-weighted MRI sequences resulted in a mean area under the curve (AUC) of 0.83, sensitivity of 0.71, and specificity of 0.78 in the cross-validation in the training cohort. The validation of the radiomics model in the external validation cohort resulted in a mean AUC of 0.80, sensitivity of 0.76, and specificity of 0.72. Lastly, validation in the prospective cohort resulted in a mean AUC of 0.91, sensitivity of 0.71, and specificity of 0.88.

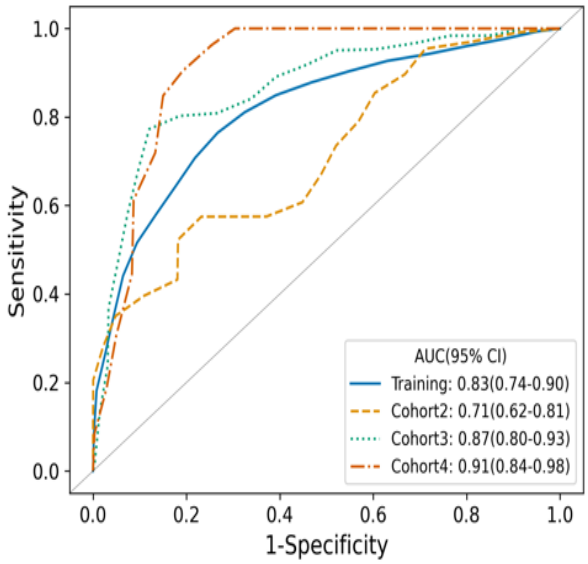
Conclusion: Our radiomics model can accurately differentiate between lipomas and ALT based on quantitative imaging features on MRI in a large, multi-center external cohort, and in prospective validation. Furthermore, our automatic and minimally interactive segmentation methods provided adequate segmentation for use in radiomics, as the need for manual segmentation was minimal. Therefore, our radiomics and segmentation pipeline provides an accurate, non-invasive, and clinically feasible method for differentiating between lipomas and ALTs, possibly limiting the need for biopsies.

References:
1. Vos M et al. Radiomics approach to distinguish between well differentiated liposarcomas and lipomas on MRI. Br J Surg. 2019 Dec;106(13):1800-1809.
2. Spaanderman D.J. et al. Minimally Interactive Segmentation of Soft-Tissue Tumors on CT and MRI using Deep Learning. 2023, Submitted.
3. Kikinis R, Pieper SD, Vosburgh K (2014) 3D Slicer: a platform for subject-specific image analysis, visualization, and clinical support. Intraoperative Imaging Image-Guided Therapy, Ferenc A. Jolesz, Editor 3(19):277–289 ISBN: 978-1-4614-7656-6 (Print) 978-1-4614-7657-3 (Online).

4. Starmans, M.P.A. et al. Reproducible radiomics through automated machine learning validated on twelve clinical applications. Medical Image Analysis. 2022 Aug; https://doi.org/10.48550/arXiv.2108.08618.
5. Starmans, M.P.A. et al. The WORC database: MRI and CT scans, segmentations, and clinical labels for 930 patients from six radiomics studies. Data in Brief. 2021 Aug; https://doi.org/10.1101/2021.08.19.21262238







Cohort 2, UC Davis, MD Anderson, UCSF and UC Irvine; Cohort 3, The Royal Marsden; Cohort 4, Erasmus Medical Center (prospective data).

Table 1. Performance of the radiomics model trained and validated on an external and prospective dataset

	Training cohort	External validation cohort (Cohort 2-3)	Prospective validation cohort (Cohort 4)
AUC	0.83 (0.75, 0.90)	0.80 (0.75, 0.85)	0.91 (0.84, 0.98)
Accuracy	0.74 (0.67, 0.82)	0.73 (0.69, 0.79)	0.83 (0.74, 0.92)
Sensitivity	0.71 (0.59, 0.84)	0.76 (0.68, 0.83)	0.71 (0.49, 0.93)
Specificity	0.78 (0.67, 0.89)	0.72 (0.65, 0.80)	0.88 (0.78, 0.97)
NPV	0.74 (0.65, 0.82)	0.77 (0.70, 0.84)	0.89 (0.81, 0.98)
PPV	0.77 (0.68, 0.86)	0.71 (0.63, 0.78)	0.67 (0.44, 0.89)

Values are mean (95% confidence interval). AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

(P 234) GENOMIC LANDSCAPE OF CLEAR CELL SARCOMA

Dawid Krzeciesa, M.Sc.[1]; Thomas Muehlenberg, PhD[1]; Susanne Grunewald, PhD[2]; Johanna Falkenhorst, MD[3]; Julia Ketzer[4]; Jonathan A. Fletcher, MD[5]; Christian Britschgi, N/A, Dr. med. Dr. rer. nat.[6]; Hans-Ulrich Schildhaus, MD[7]; Charles Keller, MD[8]; Johannes Köster, Prof. Dr. rer. nat.[9]; Stefan Fröhling, MD[10]; Sebastian Bauer, MD[3], (1) The West German Cancer Centre, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (2) West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (3) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (4) Sarcoma Center, Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (5) HarvardMedical School/Brigham and Women's Hospital, Boston, MA, (6) University Hospital Zurich, Zurich, Zurich, Switzerland, (7) Institute of Pathology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (8) Children's Cancer Therapy Development Institute, Beaverton, Oregon, Portland, OR, (9) Bioinformatics and Computational Oncology, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (10) Department of Translational Medical Oncology, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center (DKFZ), Heidelberg, Baden-Wurttemberg, Germany

Objective: Clear Cell Sarcoma (CCS) represent a rare soft tissue sarcoma with an unusually aggressive phenotype (frequent in-transit, lymphatic and early distant metastases). Translocations resulting in chimeric genes fusing EWSR1 with either ATF1 or rarely CREB1 or CREM are the defining molecular events. As of today, no effective treatment is available and 5-year survival is 59%. Bioinformatics analyses and novel disease models are urgently needed to identify and validate therapeutic vulnerabilities.

Methods: Next generation sequencing (NGS), including whole genome, exome, and transcriptome data of 23 CCS patients and three CCS cell lines were collected. Somatic copy number alterations (SCNA) were found using CNVkit [https://cnvkit.readthedocs.io/en/stable/] and GISTIC2.0 [https://broadinstitute.github.io/gistic2/], and gene fusion detection was performed using Arriba [https://github.com/suhrig/arriba]. A new variant calling approach [https://github.com/snakemake-workflows/dna-seq-varlociraptor] was optimized on a training data set using a cohort of GIST (Gastrointestinal Stromal Tumor) samples with various common and uncommon, somatic and germline variants. Click-iT EdU Flow Cytometry Cell Proliferation Assay (Thermo Fisher) was used to evaluate palbociclib response in the cell lines.

Results: Variant calling revealed few variants in cancer associated genes. SCNA analyses revealed 9p21.3 loss in 12/23 patients, and smallest region of overlap (SRO) analyses implicated CDKN2A as the principal 9p21.3 deletion target, which was also found in two out of three CCS cell lines. Arriba revealed no recurrent or apparent oncogenic gene fusions beyond EWSR1 fusions. The CDK4/6 inhibitor palbociclib had antiproliferative activity in cell lines with CDKN2A loss but not in the CDKN2A-positive subline.

Conclusion: These studies confirm EWSR1 fusion as the central genomic alteration in CCS. Recurrent loss of CDKN2A in about half of patients represents an additional genomic event. Future studies should focus on better understanding the oncogenic consequences of EWSR1 fusions, to identify therapeutic vulnerabilities.

(P 235) FUSION OF PREOPERATIVE IN SITU MAGNETIC RESONANCE IMAGING AND POSTOPERATIVE COMPUTED TOMOGRAPHY TO CORRELATE FUNCTIONAL IMAGING FEATURES AND PATHOLOGICAL CHARACTERISTICS OF SOFT TISSUE SARCOMA

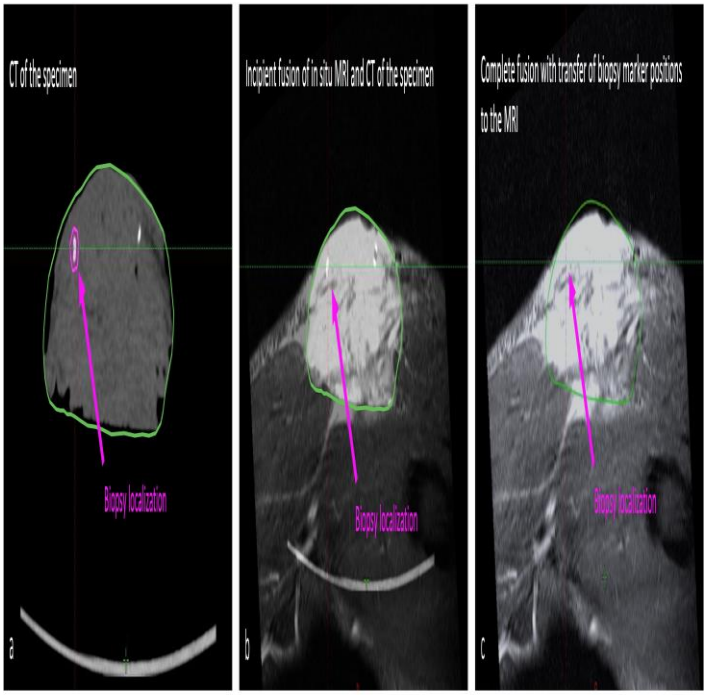
Madelaine Hettler, MD[1]; Julia Kitz, MD[2]; Ali Seif A. H., MD[3]; Manuel Guhlich, MD[4]; Babak Panahi, MD[3]; Jennifer Ernst, MD[5]; Lena-Christin Conradi, MD[6]; Michael Ghadimi, MD[6]; Philipp Ströbel, MD[6]; Jens Jakob, MD[7], (1) Department of Surgery, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, 68167 Mannheim, Germany, Mannheim, Baden-Wurttemberg, Germany, (2) Institute of Pathology, University Medical Center Goettingen, 37075 Goettingen, Germany, Göttingen, Niedersachsen, Germany, (3) Department of Diagnostic and Interventional Radiology, University Medical Center Goettingen, 37075 Goettingen, Germany, Göttingen, Niedersachsen, Germany, (4) Department of Radiotherapy and Radiation Oncology, University Medical Center Goettingen, 37075 Goettingen, Germany, Göttingen, Niedersachsen, Germany, (5) Department of Trauma Surgery, University Medical Center Hannover, 30625 Hannover,Germany, Hannover, Niedersachsen, Germany, (6) Department of General, Visceral and Pediatric Surgery, University Medical Center Goettingen, 37075 Goettingen, Germany, Göttingen, Niedersachsen, Germany, (7) University Medical Center Mannheim, University of Heidelberg, Mannheim, Baden-Wurttemberg, Germany

Objective: Treatment and prognosis of soft tissue sarcoma (STS) are primarily based on histological subtype and grading. However, due to tumor size and intratumoral heterogeneity, pretherapeutic biopsies often do not provide reliable tumor characterization. By correlating functional imaging and histopathologic features, pretreatment grading might be improved.

Methods: Twelve patients with histologically confirmed STS were included in the prospective pilot study. A diffusion weighted MRI was performed preoperatively. After tumor resection, core needle biopsies (CNBs) from distinct tumor sites from the surgical specimen were collected and a radiopaque marker was left at each biopsy site. Afterwards, a CT of the specimen visualized the markers. Fusion imaging of preoperative in situ MRI and postoperative CT was sought to evaluate the imaging characteristics of the exact sampling regions (Figure 1). The apparent diffusion coefficient (ADC) on functional MRI and histopathological grading according to Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) criteria were determined for all specimens and CNBs.

Results: Fusion imaging of pretreatment MRI and postoperative CT of the resected specimen was feasible in 7/12 cases without relevant contour discrepancies. Histological workup resulted in correct grading in 9/11 cases (81,8 %, in n=2: G1 instead of G2). Analysis of multiple tumor biopsies verified the expected intratumoral heterogeneity. Functional evaluation showed a tendency for high-grade regions (G2/G3; median (range) ± standard deviation: 1.13 (0.78–1.70) ± 0.23 × 10−3 mm2/s) to have lower ADC values than low-grade regions (G1; 1.43 (0.64–2.03) ± 0.46 × 10−3 mm2/s).

Conclusion: Our pilot study demonstrates the technical feasibility of correlating imaging features of preoperative in situ MRI and postoperative CT of the specimen by fusion imaging. In this regard, ADC appears to correlate with the FNCLCC grading criteria. Further studies are necessary to clarify the potential of functional imaging to supplement histopathological grading.



(P 236) APOBEC MUTAGENESIS IN OSTEOSARCOMA DEVELOPMENT, PROGRESSION AND RESPONSE TO THERAPY

Nuri A. Temiz, PhD[1]; Cameron Durfee, n/a[2]; Zach Seeman, n/a[1]; Reuben S. Harris, PhD[2]; David A. Largaespada, PhD[1], (1) University of Minnesota, Minneapolis, MN, (2) University of Texas Health San Antonio, San Antonio, TX

Objective: Osteosarcoma (OS) is the most common bone tumor in humans. Every year around 800-1000 cases develop in the US. OS most commonly occurs in teens and young adults with a second incidence peak in adults aged 60+. OS frequently and quickly metastasizes to the lungs. Standard combination chemotherapy uses methotrexate, Adriamycin and platinum agents (MAP therapy). In combination with surgical resection, MAP therapy has increased five-year survival from 15-20% to over 50%. However, many patients develop therapy resistance and metastasis. OS is generally immunologically cold and targeted therapies have thus far failed. While nearly all OS tumors carry TP53 mutations, there are few clear druggable genetic drivers to target. OS is characterized by massive genomic instability with many chromosomal abnormalities, but the sources of OS genomic instability are incompletely understood. APOBEC3 enzymes are cytidine deaminases that have been shown to be involved in mutating the genomes of solid tumors through a specific and context dependent mutation signature.

Methods: We have performed bioinformatic analysis of human DNA whole exome and RNA sequencing data and preliminary mouse whole genome sequencing data.

Results: The Pan Cancer Atlas Whole Genome sequencing study has observed that some bone tumors also show APOBEC mutation signature. Here, we show that APOBEC3B expression levels and its mutation signatures correlate in an OS data set. Increased

APOBEC3B levels in TARGET OS dataset shows a significant survival advantage. In other work, we have shown that APOBEC3B expression is associated with an enhanced response to platinum chemotherapy.

Conclusion: We suggest a similar synthetic lethality for APOBEC3B+ OS treated with platinum therapy. This information may help stratify OS patients. Secondly, APOBEC3B-induced mutations may include neoantigens recognizable by a nascent immune response that could be enhanced by checkpoint blockade.

(P 237) SECONDARY REARRANGEMENT CREATING A PLEC-EML4-ALK DOUBLE FUSION AS A MECHANISM OF RESISTANCE TO CRIZOTINIB IN AN EML4-ALK-POSITIVE INFLAMMATORY MYOFIBROBLASTIC TUMOR

Kurtis D. Davies, PhD[1]; Dara L. Aisner, MD, PhD[1]; Breelyn A. Wilky, MD[2]; Anthony D. Elias, MD[3]; Alessandra Maleddu, MD[1], (1) University of Colorado - Anschutz Medical Campus, Aurora, CO, (2) University of Colorado Cancer Center, Aurora, CO, (3) University of Colorado Anschutz Medical Campus, Aurora, CO

Objective: ALK rearrangement/fusion is detected in greater than 50% of inflammatory myofibroblastic tumor (IMT) cases. Inhibition of ALK fusion protein with small molecule tyrosine kinase inhibitors (TKIs) is effective in positive cases, and consequently crizotinib, a first generation inhibitor of ALK, is now regulatory agency-approved in this setting. However, acquired resistance to targeted therapy remains a clinical challenge. Resistance mechanisms to crizotinib, predominantly investigated in the setting of non-small cell lung cancer, include activation of bypass signaling, ALK kinase domain mutation, and amplification of the ALK fusion gene. Amplification and the resulting increased protein expression of the fusion is believed to reduce the ability of crizotinib to saturate its target, thus permitting enough signaling to promote tumor growth. Here we present a case of EML4-ALK-positive IMT in a 61-year-old female who demonstrated an initial response to crizotinib. Disease progression occurred following 5 cycles of crizotinib, prompting additional molecular testing to determine the mechanism of resistance.

Methods: Both the initial diagnostic sample and biopsy at time of resistance to crizotinib were assessed by the ArcherDx FusionPlex Solid Tumor next-generation sequencing-based gene fusion assay. Briefly, total nucleic acid (RNA and DNA) was extracted from enriched tumor material and submitted to library preparation using the FusionPlex kit. Libraries were sequenced via the Illumina platform and raw sequence data was analyzed by the ArcherDx analysis algorithm (v6.2.3).

Results: The initial diagnostic sample demonstrated evidence of a classic EML4-ALK fusion in which exon 20 of ALK was fused with exon 2 of EML4 (variant v5a). RNA reads extending 5' through EML4 exon 2 clearly read into the expected bases in EML4 exon 1. The same assay was performed on the sample obtained at the time of resistance. In this sample, exon 20 of ALK remained fused with exon 2 of EML4, but exon 2 of EML4 now demonstrated fusion to PLEC (several distinct isoforms were detected involving various exons of PLEC, all confirmed to be in frame). The fusion to an additional 5' gene and thus new promoter was theorized to result in increased expression (immunohistochemical studies are ongoing) that would mimic the well-described amplification of the EML4-ALK fusion as a mechanism of resistance to crizotinib. Since newer generation ALK inhibitors are more potent than crizotinib and known to overcome this resistance mechanism, the patient was initiated on alectinib therapy and demonstrated an

on-going partial response per RECIST criteria.

Conclusion: Secondary rearrangement involving EML4-ALK which places the fusion gene under control of a new promoter may be a novel mechanism of resistance to crizotinib in IMT (and potentially other ALK-fusion-driven malignancies). Alectinib, a more potent ALK inhibitor, overcame resistance in this case. More broadly, this study exemplifies the importance of molecular testing in the setting of resistance to targeted therapy to inform clinical decision making.

(P 238) EPIDEMIOLOGIC SURVEY OF MYXOFIBROSARCOM USING DATA FROM THE BONE AND SOFT TISSUE TUMOR REGISTRY IN JAPAN

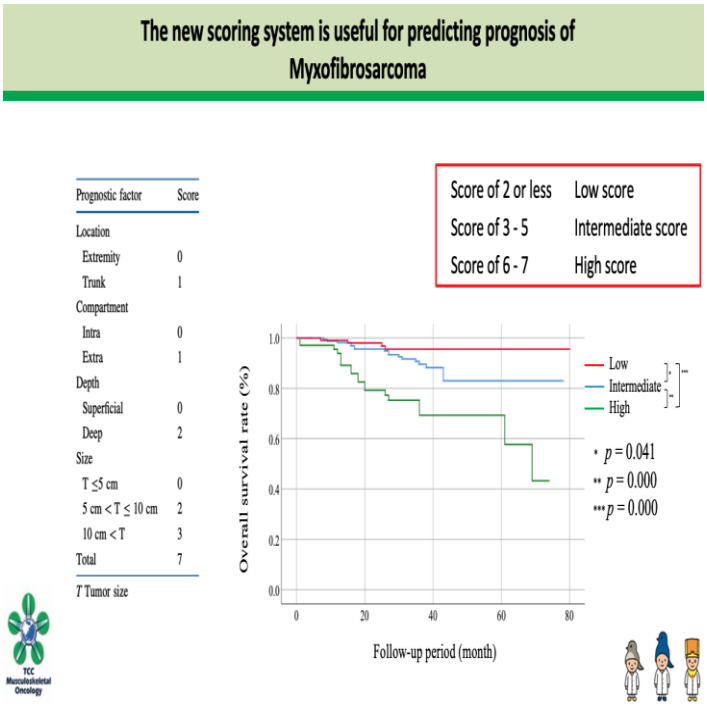
Kazutaka Kikuta, MD, PhD[1]; Satoshi Kamio, MD[2]; Akira Kawai, MD, Ph.D[3], (1) Department of Musculoskeletal Oncology and Orthopaedic Surgery, Tochigi Cancer Center, Utsunomiya, Tochigi, Japan, (2) Tochigi Cancer Center, Utsunomiya, Tochigi, Japan, (3) National Cancer Center Hospital, Tokyo, Tokyo, Japan

Objective: Myxofibrosarcoma is a common sarcoma among older patients, with locally infiltrative behavior and a predilection for local postoperative recurrence. Some studies have reported the factors affecting prognosis, although only a few have mentioned the previous staging classification systems. This study investigated the clinical overview and prognosis of myxofibrosarcoma to determine the optimal treatment.

Methods: This retrospective study analyzed the records of 349 patients with myxofibrosarcoma in the nationwide Bone and Soft Tissue Tumor Registry in Japan from 2006 to 2015. Clinical features, treatment options, and patient outcomes were investigated.

Results: Ultimately, 349 patients were identified. The overall survival rates were 93.1% at 2 years and 84.3% at 5 years. A multivariate analysis was performed using the Cox proportional hazards model. The study identified four sig- nificant prognostic factors for survival: tumor size, depth, compartment status, and location. The prognostic score was calculated by summing the scores of all the factors. The overall survival rate was 69.3% at 5 years for the patients with prognostic scores of 6 or higher. Conversely, the patients with prognostic scores of 2 or lower had a survival rate of 95.6% at 5 years.

Conclusion: Among myxofibrosarcomas, those larger than 5 cm, deep-seated, invaded into the external compartment, or in axial body parts were associated with a significantly worse prognosis. Adjuvant radiotherapy and chemotherapy did not contribute significantly to a better prognosis. Previous staging classification systems are impractical for prognosis prediction. Therefore, new classifications are needed. Further research on new treatment methods for patients with a poor prognosis will be crucial in the future.



(P 239) CANCER RADIOMIC AND PERFUSION IMAGING (CARPI) AUTOMATED FRAMEWORK: VALIDATION ON MUSCULOSKELETAL TUMORS IN A SPECIALIZED CANCER CENTER

Elvis Duran Sierra, PhD[1]; Raul F. Valenzuela, M.D[1]; Mathew Antony, M.D[2]; Colleen Costelloe, M.D[2]; Heerod Moradi, PhD[3]; John E. Madewell, M.D[1]; William A. Murphy, Jr., M.D[4]; Behrang Amini, M.D, PhD[5], (1) MD Anderson Cancer Center, Houston, TX, (2) MD Anderson cancer center, Houston, TX, (3) Texas A&M University, Houston, TX, (4) M.D Anderson cancer center, Houston, TX, (5) MD Anderson cancer Center, Houston, TX

Objective: Limitations from commercial software applications prevent the implementation of a robust and cost-efficient high-throughput cancer imaging radiomic feature extraction and perfusion analysis workflow. The purpose of this study is to develop and validate a cancer research computational solution using open-source software for vendor- and sequence-neutral high-throughput image processing and feature extraction.

Methods: The Automated Cancer Radiomic and Perfusion Imaging Framework (CARPI) is a Python-based software application that is vendor- and sequence-neutral, making it a cost-efficient and robust workflow solution for image processing of medical imaging data and tested on patient magnetic resonance imaging (MRI) sequences. The application uses contour files generated using an application of the user's choice and performs automated radiomic feature extraction and perfusion analysis. Following image processing, the application automatically populates a relational database. This workflow solution was validated using two real-world datasets, one composed of 40 pelvic chondrosarcomas and 42 sacral chordomas with a total of 82 patients, and a second validation dataset consisted of 26 undifferentiated pleomorphic soft-tissue sarcoma patients imaged at multiple points during presurgical treatment.

Results: 316 volumetric contour files and corresponding MRI series were processed using CARPI. The application performed automated extraction of 107 radiomic features from multiple MRI sequences originating from our institutional 29-magnet fleet of scanners from two manufacturers (GE and Siemens). The perfusion subroutine could also estimate seven (7) semiquantitative kinetic parameters extracted from time-intensity curves (TIC). Commercial imaging post-processing software applications available at our institution were unable to produce the same number of contours due to limitations on the types of sequences supported, licensing-related restrictions, and modality manufacturer. Furthermore, the commercial applications were limited in their capability to automatically export data and in the number of radiomic features they could extract, primarily of first-order only. For the first validation dataset, chordoma vs. chondrosarcoma, statistically significant differences ($p < 0.00047$) were found in 18 of 107 radiomic features, including six first-order and twelve high-order features from four texture classes, including Gray Level Co-occurrence Matrix (GLCM), Gray Level Dependence Matrix (GLDM), Gray Level Size Zone Matrix (GLSZM), and Gray Level Run Length Matrix (GLRLM). For the second validation dataset, UPS post-radiation (PRd), the ADC mean increased by 41% in good responders ($p=0.0017$) while firstorder_10Percentile ($p=0.0312$) provided statistically significant differences between good and partial/non-responders. The perfusion module determined that TIC type 2 and high Wash-In rate correlate with successfully treated sarcomas with 90% or more treatment-induced necrosis (TIN). In our benchmark analysis against five (5) comparable publicly available open-source software applications, our software presented the advantages of automated perfusion feature extraction, relational database generation, and graphic report export. Still, it lacks GUI and predictive model-building.

Conclusion: The CARPI processing of two clinical validation datasets has confirmed the software's ability to differentiate between different types of tumors and help predict patient response to treatment based on radiomic features. Benchmark comparison with five similar open-source solutions shows the advantages of our software in the automated perfusion feature extraction, relational database generation, and graphic report export features, although lacking a user-friendly GUI and predictive model building. In conclusion, the CARPI application provides an automated, robust, vendor-neutral, high-throughput workflow for cancer MRI data processing and radiomic feature extraction. It has the potential to expand its use to theranostic modeling development, to be generalized to other imaging modalities, and to be used to analyze clinical image datasets.

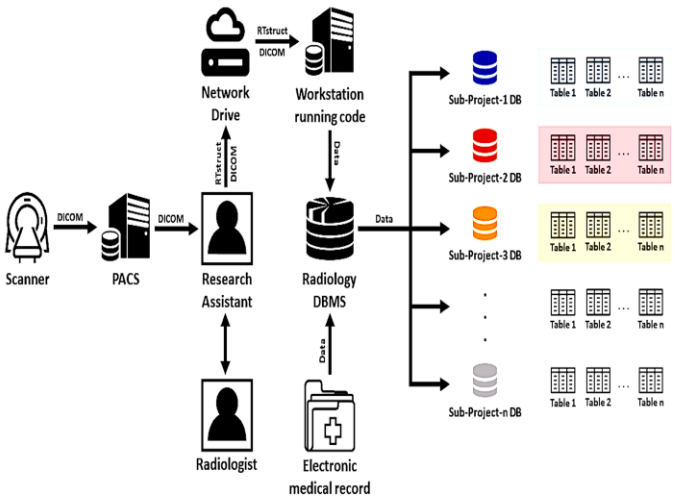


Figure 1. Summary of the workflow developed in this study. The acquired DICOM images were stored in PACS. A research assistant, in consultation with a radiologist, segmented lesions on a best-in-class segmentation application. This represents the rate-limiting and manual segment of the processing workflow. The contours (in standard RTStruct format) and corresponding DICOM files were stored on a network drive. A Python-based application on a local workstation automatically processed the images and stored the data in the radiology database management system (DBMS). Clinical data from the electronic medical record can be included in the radiology DBMS and used for various sub-projects based on subsets of the data.

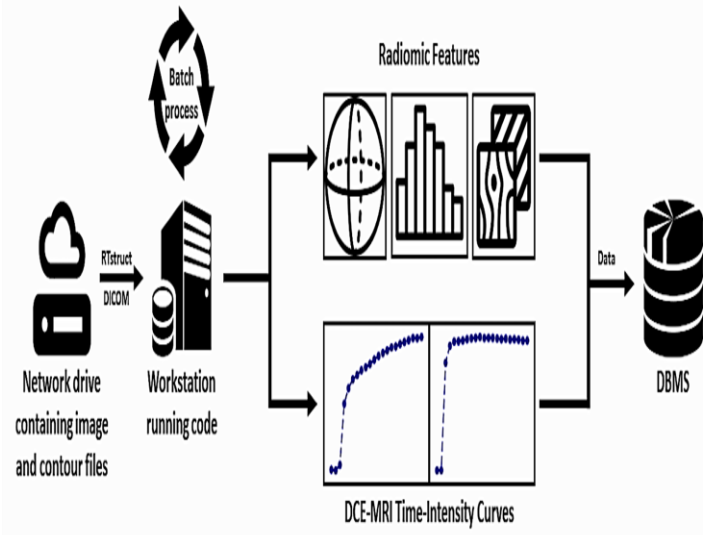


Figure 2. Schematic summarizing the automated feature extraction workflow. Contour (RTStruct) and associated image (DICOM) files from multiple patients across multiple time points were retrieved from a secure internal network drive and batch processed using our Python-based application. Radiomic features and perfusion estimates from time-intensity curves were computed and stored in the DBMS.

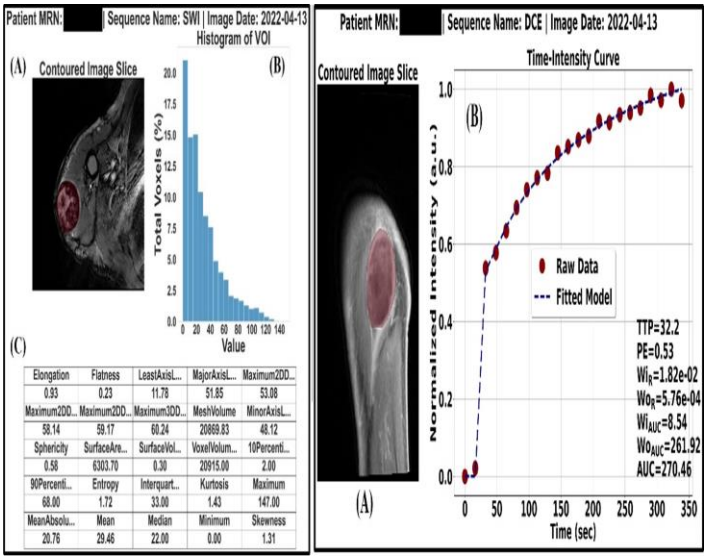


Figure 3. Representative graphic reports from a UPS patient imaged post-radiation that were automatically generated by CARPI. Left panel: Report of an SWI image showing the (A) 2D contoured tumor from a representative image slice, (B) histogram of VOI intensity, and (C) table with 25 out of 107 radiomic features extracted from the VOI. Right panel: Report of DCE image showing the (A) 2D contoured tumor from a representative image slice, and (B) time-intensity curve plot and 7 semiquantitative kinetic parameters extracted from the fitted model.

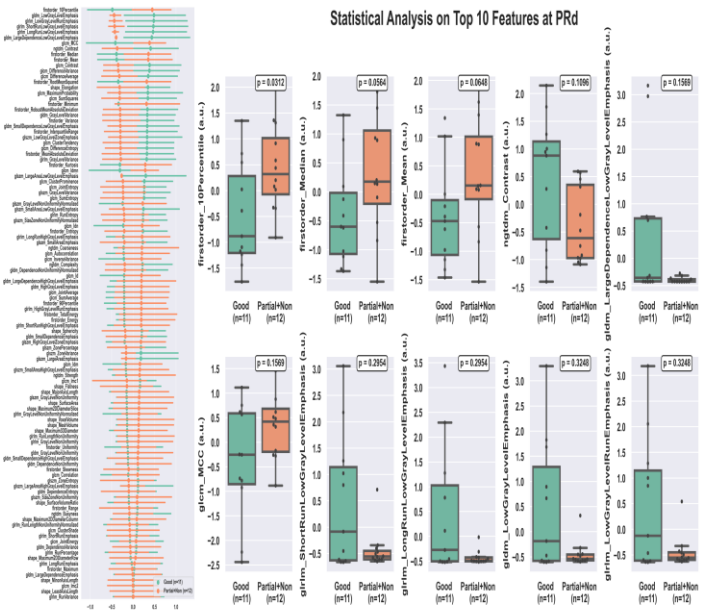


Figure 4. UPS ADC analysis at post-radiation (PRd): Scatter plot (left panel) showing the mean and 95% confidence interval for each of the 107 radiomic features extracted comparing good responders vs. partial/non-responders at post-radiation. Features are sorted from top to bottom based on the difference between group means. Boxplots of top 10 radiomic features (right panel) comparing good vs. partial/non-responders. P-values resulted from two-tailed non-parametric Wilcoxon rank-sum tests.

(P 240) IDENTIFYING PATIENTS AT HIGH RISK OF HIP FRACTURE DUE TO BONE TUMORS BY COMPUTATIONAL BIOMECHANICS: A RANDOMIZED PROSPECTIVE CLINICAL STUDY

Amir Sternheim, Dr[1]; Omri Merose, MD[2]; Ortal Segal, MD[3]; Solomon Dadia, MD[2]; Yair Gortzak, MD MSc[3]; Aharon Feinstone, Prof[4]; Gal Ariel, Dr[5]; Gal Dahan, PhD[5]; Zohar Yosibash, Prof.[4], (1) National Unit of Orthopaedic Oncology, Tel Aviv Medical Center, Tel Aviv University, Tel Aviv, Tel Aviv, Israel, (2) Tel Aviv Medical Center, Tel Aviv, Israel, TEL AVIV, Tel Aviv, Israel, (3) Tel Aviv Medical Center, Tel Aviv, Israel, TEL AVIV, HaMerkaz, Israel, (4) Tel Aviv University, Tel Aviv, Tel Aviv, Israel, (5) Tel Aviv Medical Center, Tel Aviv, Tel Aviv, Israel

Objective: The low sensitivity of Mirels' score poses challenges in predicting fracture risk in patients with metastatic tumors in the femur. However, there is a promising quantitative patient-specific decision support tool called Simfini-TUMOR, which is an autonomous CT-based finite element analysis system (CTFEA) [2,3]. This study evaluates the clinical performance of CTFEA in a prospective randomized trial.

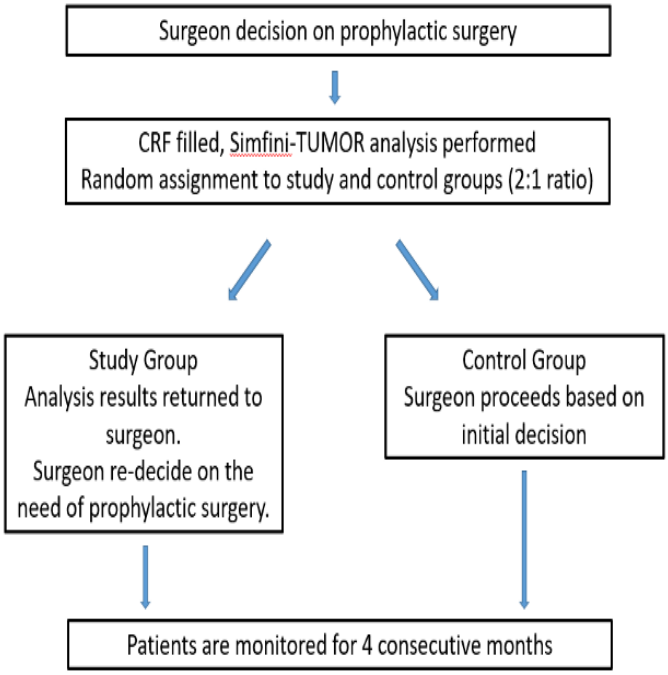
Methods: Patients with femoral metastatic tumors who were referred to the orthopedic oncology department were included in the study. Two orthopedic oncology surgeons assessed the need for prophylactic surgery, and CTFEA analysis was conducted to evaluate the fracture risk. In the study group, the surgeons were provided with the CTFEA analysis report containing a quantified fracture risk to aid their final decision-making. In the control group, the surgeons were unaware of the CTFEA results, and patients received treatment based on the initial surgeons' recommendation. The randomization ratio between the study and control groups was 2:1. All patients were followed up for a duration of four months. The study flowchart can be found in Figure 1. A total of 110 patients were enrolled in the study between 2017 and 2023, and a statistical analysis was conducted. Each femur underwent a simulated stance position loading CTFEA with a force of 2.5 times the body weight applied at the femur's head to determine the fracture risk.

Results: 98 CTFEAs were successfully completed. 12 CTs were excluded due to either implants in the other femur or those who did undergo a surgery but could not be followed for 4 months (died).
The study group contains 64 patients (37 Females, 27 males), ages 31-89, mean 64, STD 13.4.
The control group contains 34 patients (17 females, 17 males), ages 36-83, mean 64. STD 11.8. Most common tumors types were breast (26%) and prostate (13%).
The CTFEA was completed successfully in 90% of the cases.
In the study group, 34 surgeries (53%) were performed whereas in the control group 19 surgeries (56%)
In 95% of the cases where the CTFEA predicted low risk of fracture (and thus recommended not to perform surgery), the patient did not fracture the femur during the following 4 months.
In the study group there were two fractures (3%) – one month and two and a half months after the CT scan, in both cases the analysis predicted high risk of fracture in the fractured regions.
In the control group there was one fracture (3%) 6 months after the CT scan, caused by a fall.

Conclusion: The preliminary results show that CTFEA is a valuable tool in the clinical decision making process, and is able to assist the surgeon in making the best treatment choice for the patient. In two recorded cases of fractures CTFEA suggested a high risk of fracture in the correct regions.
In the study group the surgeons decided not to perform a prophylactic surgery, whereas the CTFEA indicated a high risk of fracture. Following CTFEA results a new X-ray showed a crack

initiation in femur's neck, the patient underwent immediately a fixation procedure, avoiding a complicated fracture to occur.
In many cases CTFEA results strengthens the clinical decision.

References
[1] Yosibash Z, Myers K, Trabelsi N, Sternheim A, "Autonomous FEs (AFE) - A stride toward personalized medicine", Comp. Math. Appl., 80(11), pp. 2417-2432, 2020.
[2] Sternheim A, Giladi O, Gortzak Y, Drexler M, Salai M, Trabelsi N, Milgrom C and Yosibash Z, "Pathological fracture risk assessment in patients with femoral metastases using CT-based finite element methods. A retrospective clinical study", Bone, 110, pp. 215-220, 2018.
[3] Sternheim A, Traub F, Trabelsi N, Gortzak Y, Dadia S, Snir N, Gorfine M and Yosibash Z., "When and where do patients with bone metastases actually break their femur? A CT-based finite element analysis of patients", Bone & Joint Jour., 102-B, No. 5, pp. 638-645, 2020.



(P 241) ASSOCIATION OF ADC VALUES WITH DESMOID TUMOR SHRINKAGE DURING ACTIVE OBSERVATION

Masato Sugawara, MD, PhD; Daiichiro Takahara, M.D.; Chiomi Shirai, M.D.; Michiaki Takagi, M.D., Ph.D., Yamagata University Faculty of Medicine, Department of Orthopaedic Surgery, Yamagata, Yamagata, Japan

Objective: Although recent guidelines recommend wait-and-see policy for desmoid tumor (DT) as first management, it is difficult to predict the clinical course after initial diagnosis. Magnetic resonance imaging (MRI) is often used to evaluate the status of DT, and association between T2 signal intensity and progression during active observation has been reported. Apparent-

diffusion-coefficient (ADC) value is quantitative parameter of diffusion-weighted-imaging (DWI) which reflects diffusion movements of water molecules in various tissues. Although changes in cellularity or fibrosis in DT may affect the diffusion of water molecules in the tissue, it is not clear if there is a relationship between changes in ADC values and the clinical course during active observation of DT. We investigated whether changes in ADC values are related to the clinical course of DT.

Methods: Eleven patients diagnosed as DT in our hospital between April 2013 and August 2022 with measurable ADC values on MRI were included in the study. There were 7 male and 4 female patients with a median age of 49.1 years (range, 17-78) at the time of initial diagnosis. The mean follow-up period was 37 months (range, 3-78). The origin sites were 5 abdominal and 6 extra-abdominal. The mean diameter of tumor at the first visit was 5.2 cm (range, 2.4-9). Tumors that continued to grow in size compared with the initial diagnosis were designated as group G, and those that eventually shrank in size at final periods were designated as group S. For these two groups, the tumor size that defined as the sum of the longest diameters of the horizontal section and the ADC value were investigated at the time of initial examination (period A), at the time of the greatest enlargement (period B), and at the time of the greatest shrinkage (period C), and their associations were statistically analyzed.

Results: There were 5 patients in group G and 6 patients in group S. In group G, the mean size (cm) at period A and B were each 10.2 and 15.3, and the mean ADC value (×10-3 mm2/sec) were each 1.525 and 1.575, with no significant difference. In group S, the mean size at period A, B and C were each 7.4，9.2 and 4.7, and the mean ADC values were each 1.511, 1.602, and 1.139.　There was a significant difference in the change in ADC values between at period A and C (p=0.024) and between at period B and C (p=0.033) (repeated-measures-ANOVA and Holm–Bonferroni method) (Figure 1). There were no significant differences in ADC values at period A or B between the group G and S or within each group （Figure 2）.

Conclusion: The ADC value is a numerical expression of the diffusivity of water molecules, with higher values indicating easier diffusion and lower values indicating harder diffusion. DT become lower signal than muscle on T2-weighted images with shrinkage, suggesting that the increase in fibrous matrix during shrinkage prevents diffusion of water molecules, resulting in a lower ADC value. Although this study could not demonstrate that ADC values are a predictor of shrinkage or enlargement, further studies are needed to confirm this hypothesis in more cases.

(P 242) WHOLE BODY PET/MRI FOR STAGING OF MYXOID LIPOSARCOMA

Lukas M. Nystrom, MD[1]; Cong Fan, BA[2]; Kevin Y. Zhu, BS[3]; Hakan Ilaslan, MD,MBA[4]; Zachary D. Burke, MD[5]; Nathan W. Mesko, MD[1], (1) Cleveland Clinic, Cleveland, OH, (2) Cleveland Clinic Lerner College of Medicine, Cleveland Heights, OH, (3) The Cleveland Clinic Foundation, Cleveland Heights, OH, (4) Cleveland clinic, Cleveland, OH, (5) Celveland Clinic Sarcoma Program, Cleveland, OH

Objective: Myxoid liposarcoma (MLS) has a unique predilection for metastasis to rare sites. The National Comprehensive Cancer Network (NCCN) recommends staging with computed tomography (CT) of the chest, abdomen, and pelvis and magnetic resonance imaging (MRI) of the entire spine. This study compares the performance of whole body scanning using positron emission tomography (PET) plus MRI and/or CT with NCCN guidelines.

Methods: A retrospective case series was conducted including patients with biopsy proven MLS from2015 to 2022. Patients were excluded if they 1) did not complete staging, or 2) had their care outside our institution. Patients were separated into cohorts based upon whether they were staged initially with whole body PET/MRI, or standard NCCN staging regimen of CT chest/abdomen/pelvis and whole spine MRI. We recorded detection of initial metastases, standardized uptake value (SUV) of primary tumor and metastatic lesions, and development of local recurrence and/or new metastases. Kruskal-Wallis and Fisher's exact test were used to compare continuous and categorical variables, respectively.

Results: In total 31 patients with MLS were identified who had complete staging. 21 of these had staging completed with whole body PET/MRI or PET/CT, and the other 10 had staging with standard NCCN approach. Three (14.3%) patients in the PET group had metastatic disease on initial presentation and no initial metastases were identified in the NCCN group (Table 1). The three patients with initial metastatic disease had a total of 6 metastatic locations, all of which would have been detected by NCCN guidelines. Two patients in the initial NCCN group had new metastases discovered by CT chest or MRI spine. Three patients in the initial PET group had new metastases discovered by whole body PET/MRI or MRI spine. As shown in Table 1, the SUV of primary and metastatic lesions was 3.6 and 3.2, respectively.

Conclusion: Our data indicate that whole-body PET modalities for staging MLS appears to detect initial and new metastatic disease as well as current NCCN guidelines. The added value of the PET is questionable given the very low SUV of primary and metastatic lesions. Whole body staging, with or without PET, offers the advantages of a simplified and expedited staging regimen. Given that the numbers in this study are low, it is important to study this topic further either in a collaborative study design.

	NCCN Group	PET Group	p
n	10	21	
Histologic Grade (%)			0.581
1	6 (60.0)	14 (66.7)	
2	4 (40.0)	5 (23.8)	
3	0 (0.0)	2 (9.5)	
Initial Metastatic Disease = Yes (%)	0 (0.0)	3 (14.3)	0.533
New Metastatic Disease = Yes (%)	2 (20.0)	3 (14.3)	1
Time to New Mets (median [IQR])	610.50 [553.25, 667.75]	419.00 [414.00, 479.50]	0.248
SUVMax of Initial Mets (median [IQR])	*NA [NA, NA]*	3.60 [3.55, 3.80]	*NA*
SUVMax of New Mets (median [IQR])	2.55 [2.08, 3.02]	3.20 [2.75, 3.65]	0.439

Table 1. Tumor characteristics, presence of initial and new metastatic disease in both the NCCN and PET imaging groups.

(P 243) EVALUATION OF THE USE OF WGS IN ROUTINE DIAGNOSTICS FOR BONE AND SOFT TISSUE TUMORS

Karin Wallander, MD, PhD[1]; Ingegerd Ofverholm, MD, PhD[2]; Yingbo Lin, PhD[3]; Cecilia Haglund, MD[3]; Venkatesh Chellappa, MS[3]; Panagiotis Tsagkozis, MD, PhD[1]; Asle C. Hesla, MD, PhD[4]; Andri Papakonstantinou, MD, PhD[3]; Robert Bränström, Adjunct senior lecturer, MD[3]; Valtteri Wirta, PhD[5]; Markus Mayrhofer, PhD[3]; Johan Lindberg, PI[3]; Felix Haglund de Flon, MD, Ph.D[6], (1) Karolinska Institutet, Stockholm, Stockholms Lan, Sweden, (2) Oncology Pathology, Stockholm, Stockholms Lan, Sweden, (3) Karolinska institutet, Stockholm, Stockholms Lan, Sweden, (4) Karolinska University Hospital, Sollentuna, Stockholms Lan, Sweden, (5) SciLifeLab / Karolinska institutet, Solna, Stockholms Lan, Sweden, (6) Karolinska University Hospital, Stockholm, Stockholms Lan, Sweden

Objective: Molecular genetics may aid in the classification of soft tissue and bone tumors. Many different methods are currently in clinical practice. With technological advancement, comprehensive sequencing (WGS) has become economically feasible, but its value and role in the routine setting is yet to be determined. We aimed to evaluate the clinical impact of introducing whole-genome sequencing (WGS) in routine diagnostics for all sarcoma cases referred to Sarcoma Center Karolinska, Sweden's largest tertiary center for sarcomas. Primary endpoints included if WGS would change or confirm the histopathological diagnosis set clinically, and if germline analysis would detect hereditary cancer syndromes not identified by the clinicians.

Methods: In this prospective single-center study, we implemented WGS as a component of the diagnostic evaluation for patients presenting with bone or soft tissue tumors suspicious of sarcoma. We performed DNA sequencing on tumor and normal samples obtained from fresh frozen tissue and peripheral blood respectively. Sequencing was conducted once per week using the Illumina NovaSeq platform. To analyze the genomic data, we employed BALSAMIC and MIP bioinformatics tools, along with the in-house developed AutoSeq Informatics framework. Somatic single nucleotide variants, structural rearrangements, and copy number alterations were examined by clinical pathologists and geneticists. Assessment of germline variants followed the ACMG criteria, and pathogenic variants were addressed in accordance with local clinical guidelines.

Results: In this first phase of the study, 200 patients have been included. WGS altered the diagnosis in 17 patients (corresponding to 7% of high-grade sarcomas, 0% of low-grade sarcomas, 20% of benign mesenchymal tumors, 33% of non-mesenchymal tumors, and 16% of non-neoplastic lesions). Treatment prognostic targets (Tier 1-2, excluding MDM2 amplification) were identified in 27/29 GISTs, 8/95 high-grade sarcomas and 1/22 low-grade sarcomas. The preoperative diagnosis had significant inaccuracy in 14 cases (6-14% depending on setting) when compared to the final histopathological diagnosis. If WGS had been performed on preoperative material, the genomic data could have corrected 11 of the 14 faulty diagnoses (79%). Notably, 8 cases had diagnostic pitfalls (non-subtype specific genetic variants or fusions). In the germline analyses, 14% of the patients were found to have a theoretically clinically actionable variant, including pathogenic variants in the genes TP53, RB1, BRCA1, KCNQ1, MLH1, FH, or CHEK2. Out of those, 80% were associated with a known hereditary cancer syndrome.

Conclusion: WGS can become an important complementary tool for sarcoma diagnostics if it is used correctly. Germline analysis can preferably be added in parallel, to screen for hereditary

cancer syndromes.

(P 244) A DEEP FEW-SHOT META-LEARNING STRATEGY FOR AUTONOMOUS CLASSIFICATION OF BONE METASTASIS IN RADIONUCLIDE BONE SCANS

Ahmad P. Tafti, PhD[1]; Nickolas Littlefield, MS[1]; Luke Carlson, MS[1]; Matthew F. Gong, MD[1]; Soheyla Amirian, PhD[2]; Ines Lohse, PhD[1]; Kurt R. Weiss, MD[1], (1) University of Pittsburgh, Pittsburgh, PA, (2) University of Georgia, Athens, GA

Objective: Automatic classification of bone metastasis using only radionuclide scans will facilitate communication among radiologists and surgeons, offering a standardized approach to cope with artificial intelligence (AI)-powered medical image analysis. The objective of this work is to present a novel deep few-shot meta-learning strategy for the autonomous classification of bone metastasis in radionuclide bone scans. The primary aim is to address the challenges posed by limited labeled data by leveraging the power of meta-learning, enabling the model to quickly adapt to new classes with only a few sample images. Our approach combines deep neural networks and meta-learning techniques to achieve high accuracy and robustness in classifying bone metastasis, even with scarce labeled data. This work can facilitate early and accurate diagnosis, as well as aid in personalized treatment planning for cancer patients where the gold-standard classification required for training is lacking or expensive to achieve.

Methods: Deep learning methods generally require large number of images to learn a feature representation from medical images. We implemented a deep few-shot meta-learning pipeline using prototypical networks to perform both 5-way 10-shot and 5-way 25-shot classification. The goal is to be able to quickly adapt and automatically predict bone metastasis in unseen classes throughout different regions of the body. Various meta-learning tasks were created for training, validation, and testing with no overlapping regions in each split with 18 different classes in training, and 9 different classes in both validation and testing, with all across chest, head, knee, pelvis, shoulder, and vertebra. All images and the class labels were collected and constructed from the open-access BS-80K dataset (DOI: 10.1016/j.compbiomed.2022.106221).

Results: For 10-shot 5-way classification and 25-shot 5-way classification, 500 test tasks were randomly sampled. The average accuracy (AUC) for 10-shot 5-way classification was 0.928 +/- 0.085, while the accuracy for 25-shot 5-way classification was 0.932 +/- 0.081.

Conclusion: Based on the experimental results, we conclude that our deep few-shot meta-learning strategy exhibits promising performance in the autonomous classification of bone metastasis in radionuclide bone scans, offering potentials of quickly classifying new unseen images using only a few labeled images. This is a viable approach in settings where the gold-standard images required for training is lacking or expensive to achieve in a clinical setting. While our work in the future will further focus on the implementation and uptake of this approach, we will aim to incorporate multiple clinical data into this pipeline to facilitate personalized treatment planning for cancer patients.

(P 245) IN VIVO USE OF RAMAN SPECTROSCOPY IN SOFT TISSUE SARCOMA RESECTION

Jean-Philippe Dulude, MD[1]; Hugo LeMoël, B.Sc.[2]; Frédérick Dallaire, Ph.D.[2]; Josee Doyon, Dr[3]; Georges Basile, MD, FRCSC[4]; Frédéric Leblond, Ph.D.[2]; Mai-Kim Gervais, MD, FRCSC[4], (1) Université de Montréal, Montréal, PQ, Canada, (2) Polytechnique Montréal, Montreal, PQ, Canada, (3) CIUSSS Est-de-l'Île-de-Montréal, Montreal, PQ, Canada, (4) Hôpital Maisonneuve-Rosemont, Montreal, PQ, Canada

Objective: Negative-margin resection is one of the most significant prognostic factors for retroperitoneal soft tissue sarcoma (STS) survival. Raman spectroscopy is an optical analysis technique based on interaction between fixed-wavelength photons and tissue samples. It provides information on human tissue samples at a molecular level, in a non-destructive manner, and sufficiently quickly not to disrupt surgical workflow. Neoplastic transformation of a healthy tissue involves important biomolecular changes that generate significant changes in the tissue's corresponding Raman spectrum. Machine learning classification algorithms can detect these spectrum modifications to classify a given sample as tumoral or healthy tissue. This study aims to determine the sensitivity, specificity, and accuracy of a Raman spectroscopy handheld point probe paired with a random forest machine learning classification algorithm in detecting soft tissue sarcoma in an in vivo environment during STS resection.

Methods: Patients were recruited amongst those referred to Maisonneuve-Rosemont Hospital (Montreal, QC) from January 2021 to June 2023 for retroperitoneal and extremity STS resection. Raman spectra were acquired during STS resection using a Raman handheld point probe (UltraProbe), developed in collaboration between the Laboratory of Radiological Optics (LRO) from Polytechnique Montréal and EmVision LLC. Spectra were acquired from healthy adipose, muscular, and fibrotic tissue in vivo. Spectra were acquired from the sarcoma specimen following resection. For every spectrum acquired, a tissue sample was resected and examined by an expert pathologist to establish a histopathological diagnosis. Every spectrum was added to a database along with its associated histopathological diagnosis. Using this database, sequential binary machine learning random forest classification algorithms were developed and trained to predict the diagnosis associated with new Raman spectra: soft tissue sarcoma or healthy tissue. Spectra classification was done in two steps, the first being to perform a binary classification to predict whether the spectrum of interest has a high lipid content or not using a random forest machine learning classification algorithm. A second classification followed to classify high-lipid content Raman spectra as healthy adipose tissue or liposarcoma. For spectra classified as non lipidic, a multiclass classifier was built to classify as healthy tissue or other sarcoma subtypes. Sensitivity, specificity, and accuracy calculations were made to compare the classifier's prediction to the histopathological diagnosis used as a gold-standard.

Results: 29 patients have been recruited in the study. Preliminary results following complete histopathological diagnosis and spectra processing are available for 9 participants. The preliminary dataset includes 332 labeled Raman spectra. Well differentiated liposarcoma was the only sarcoma subtype with sufficient spectra for classification. The classification of Raman spectra between lipidic and non-lipidic tissue was performed with a sensitivity of 97%, a specificity of 91%, and an accuracy of 95%. The second classification of lipidic spectra as well differentiated liposarcoma or healthy adipose tissue was performed with a sensitivity of 93%, a specificity of 95%, and an accuracy of 94%.

Conclusion: The presented results are from a preliminary dataset. Full results will be available

in November 2023. The presented results show that Raman spectroscopy can be used to detect well differentiated liposarcomas in an in vivo setting. Its sensitivity, specificity and accuracy were greater than 90%. Raman spectroscopy is an optical tissue analysis technique that is fast, precise, non-destructive, and can thus be repeated as needed during sarcoma resection surgery without disrupting the surgical workflow. Further studies will be needed to determine if its use can lead to a reduced rate of microscopically positive margin resections.

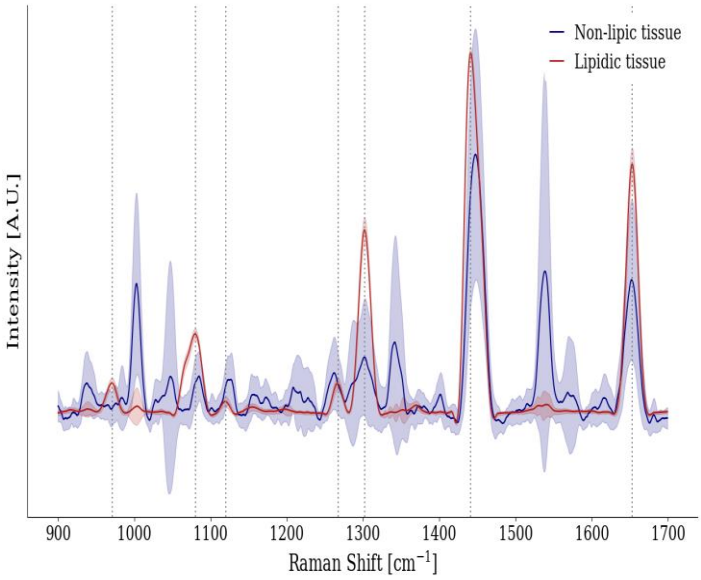


Figure1 : Average Raman spectra with shaded standard deviation of lipidic tissue (healthy adipose tissue, well differentiated liposarcoma, and dedifferentiated liposarcoma) and non-lipidic tissue. Dotted lines indicate the location of the main Raman peaks of healthy adipose tissue.

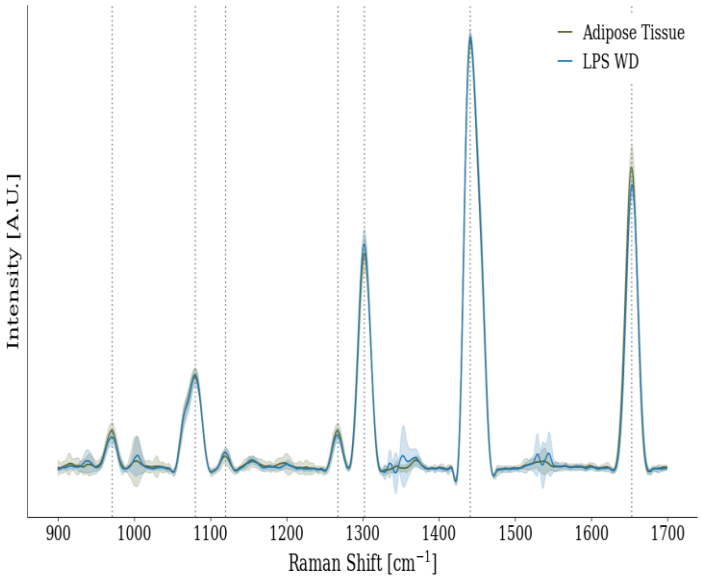


Figure2 : Average Raman Spectra with shaded standard deviation of healthy adipose tissue and well differentiated liposarcoma (LPS WD) samples. Dotted lines indicate the location of the main Raman peaks of healthy adipose tissue.

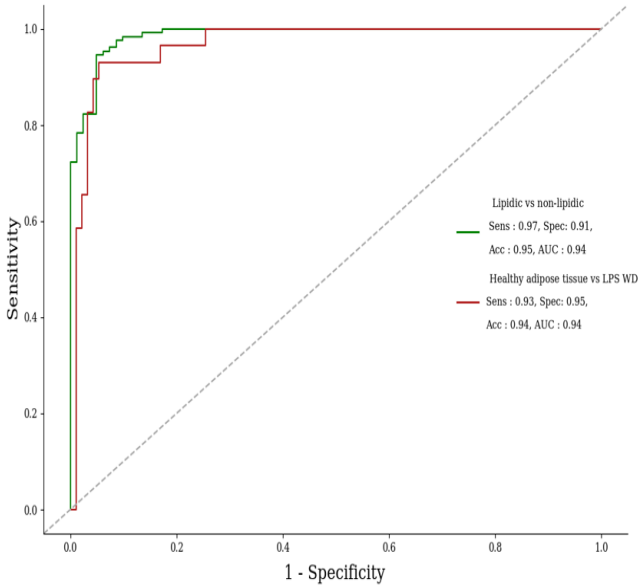


Figure3 : ROC curves of random forest classifiers of lipidic vs non-lipidic tissue, and LPS WD vs healthy adipose tissue.

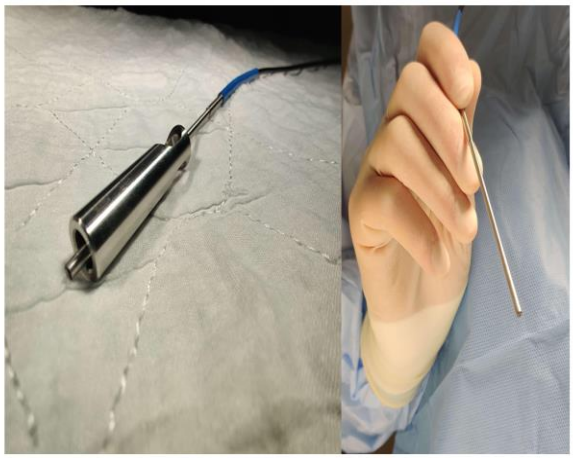


Figure4 : The Raman spectroscopy hand-held UltraProbe (EmVision LLC) used for acquisition of Raman Spectra during STS resection surgery.

Preclinical Models & Translational Research

(P 246) IMMUNOCOMPETENT MODELING OF PLEOMORPHIC LIPOSARCOMA

Jeffrey Brown, MD[1]; Kevin B. Jones, MD[2], (1) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, (2) University of Utah, Salt Lake City, UT

Objective: Introduction: Soft-tissue sarcoma represents an uncommon and heterogenous group of tumors of mesenchymal origin that carries a mortality burden incommensurate with its prevalence. Local treatment largely consists of radiation therapy and wide surgical resection, but options for systemic therapy in the setting of metastatic disease are limited and largely ineffective, prompting exploration of novel therapeutic strategies and experimental models. As with other cancers, sarcoma cell lines and patient-derived xenograft models have been developed and used to characterize these tumors and identify therapeutic targets, but these models have inherent limitations. The establishment of genetically-engineered mouse models represents a more realistic framework for reproducing sarcoma pathologic and therapeutic conditions. Pleomorphic liposarcoma is a rare sub-group of soft-tissue sarcoma with complex genomic features, frequently involving Tp53 and Rb1 mutations.

Methods: Methods: Tp53fl/fl/Rb1fl/fl/ Ptenfl/fl (RPP) mice were used to reliably generate an immunocompetent model of mouse pleomorphic liposarcoma through Cre-mediated conditional silencing of the Tp53, Rb1, and Pten tumor suppressor genes. Evaluation of tumor-infiltrating lymphocytes was assessed with immunostaining for CD4, CD8, and PD-L1, and flow cytometry with analysis of CD45, CD3, CD4, CD8, CD19, F4/80, CD11b, and NKp46 sub-populations.

Results: Results: Mice reliably produced noticeable soft-tissue tumors in approximately 12 weeks with rapid tumor growth between 12 and 20 weeks of life, after which mice reached euthanasia criteria. Histologic features were consistent with pleomorphic liposarcoma, including widespread pleomorphic lipoblasts. Immunostaining and flow cytometry demonstrated scant tumor infiltrating lymphocytes.

Conclusion: Conclusion: A genetically engineered RPP mice reliably produced soft-tissue tumors consistent with pleomorphic liposarcoma, which immunological findings consist with other soft-tissue sarcomas. This model may demonstrate utility in testing treatments for this rare disease, including immunomodulatory therapies.

(P 247) HARNESSING EWING SARCOMA'S REPLICATION STRESS BY TARGETING DDK AND WEE1 IN COMBINATION

Ajay Gupta, MD, MS[1]; Bryan Gillard, B.S.[2]; Kristopher Attwood, PhD[2]; Jeffrey Martin, PhD[2]; Joyce Ohm, PhD[2], (1) Roswell Park Oishei Children's Cancer and Blood Disorders Program, Buffalo, NY, (2) Roswell Park Comprehensive Cancer Center, Buffalo, NY

Objective: Ewing sarcoma is an aggressive pediatric cancer for which chemotherapy is very intense and often results in acute toxicities. Here, we have found that the combined inhibition of CDC7 kinase (DDK) and WEE1 kinase forces high levels of mitotic errors and synergistic cell death, specifically in Ewing sarcoma cells. Furthermore, when DDK and WEE1 inhibitors are used with irinotecan in murine models, the combination results in impressive reduction of tumor burden. This therapeutic strategy has the potential to provide a highly efficacious and minimally toxic treatment strategy for patients with Ewing sarcoma.

Methods: A673 (Ewing; ATCC, catalog no. CRL-1598, RRID:CVCL_0080) and RD-ES (Ewing; ATCC catalog no. HTB-166, RRID:CVCL_2169) cell lines were obtained through ATCC. TC32 (Ewing; RRID:CVCL_7151) were obtained through Children's Oncology Group. A673 cells were cultured in DMEM, RDES, and TC32 in RPMI1640. All media was supplemented with 10% FBS and 1% antibiotic-antimycotic (Gibco Life Technologies). All cell lines were grown at 37°C and 5% CO2 and were used within 10 passages of receipt from ATCC or COG. Cells were tested for Mycoplasma and all Mycoplasma testing was conducted by PCR through IDEXX BioAnalytics and was negative. Cell lines authentication was performed via short tandem repeat (STR) profiling.

Cells were treated with 300 nmol/L TAK-931 (DDK inhibitor), 1 μmol/L MK1775 (WEE1 inhibitor) or combination for 24 hours. Cells were then fixed and stained for DNA content (PI) and pHH3 and analyzed via FACS. Premature mitotic cells were identified as cells that stained positive for pHH3 that contain < 4N DNA content. Quantification of percentage of premature mitotic cells as a proportion of total mitotic cells [(premature mitotic cells/total mitotic cells) * 100%] (n = 3 biological replicates, two-way ANOVA, ****, $P < 0.0001$).

Mice were housed in the Laboratory Animal Shared Resource (LASR) at Roswell Park in a limited access barrier facility. LASR is an AAALAC International Accredited Animal Program. Female NSG mice at 6 to 8 weeks of age received subcutaneous injection of 1 million TC32 cells in the left flank. Two times weekly calipers used to measure change in tumor growth. Treatments started once tumors reached 200 mm3. And were performed for 3 weeks. In the second experiment, mice were rechallenged with another 3 weeks of treatment at Week 6. Mice were treated with either no therapy (vehicle control – 5 mice), MK1775 (Wee1 inhibitor; 50 mg/kg PO daily Days 1-5) + TAK-931 (DDK inhibitor; 60 mg/kg PO daily Days 1-3) (DDKi + WEE1i - 10 mice), Trabectedin (0.15 mg/kg IV or IO weekly x 3 weeks) (10 mice), Irinotecan (15 mg/kg IV weekly) + Temozolomide (40 mg/kg IP weekly) (standard relapse comparator - 10 mice), Irinotecan + MK1775 (10 mice), Cyclophosphamide (40mg/kg IV) + Doxorubicin (3.3mg/kg IV) + Vincristine (0.5mg/kg IV) given on Day 1 once on day 1 of week 1 (standard up-front regimen – 10 mice). Irinotecan + MK1775 + TAK-931 (novel triplet - 10 mice). Of note, the DDK and WEE1 inhibitors were dosed BID (twice daily) in the first experiment and then changed to daily in the second experiment. If tumors reached volume greater than 2000 mm3, the mice were euthanized as per Roswell Park Institutional Animal Care and Use Committee (IACUC) guidelines.

The survival data were summarized by treatment group using Kaplan-Meier curves, where estimates of the median time were obtained with 95% confidence intervals. Associations were assessed using the log-rank test. All analyses were conducted in SAS v9.4 (Cary, NC) at a significance level of 0.05.

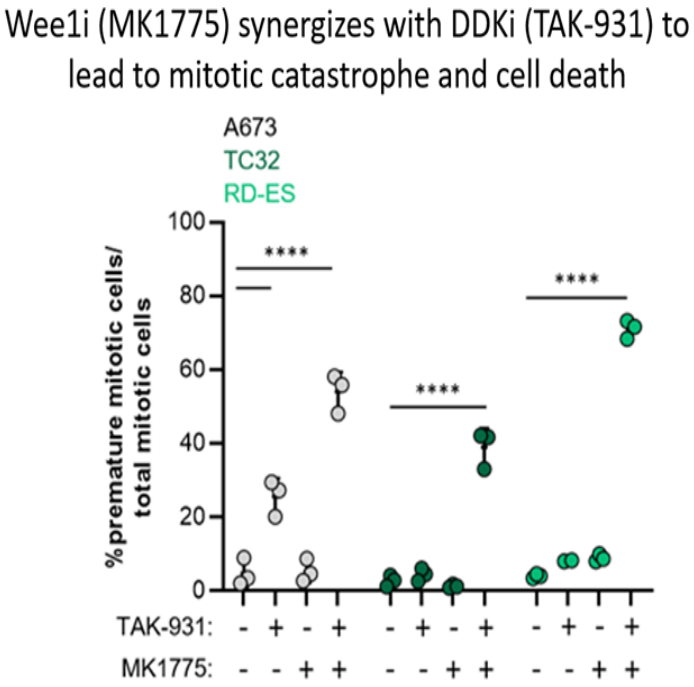
Results: The inhibition of DDK in Ewing sarcoma cells results in a dramatic reduction in the rate of DNA replication, significantly delaying replication completion and forcing a mitotic arrest to maintain viable levels of genomic integrity. The mitotic arrest is highly dependent on the activity of the checkpoint kinase WEE1. This stress places a heavy burden onto WEE1, making

it a prime target for therapeutic intervention in this context. Our in vitro data confirms that the combined inhibition of WEE1 and DDK results in the unscheduled mitotic entry of Ewing cells with underreplicated DNA (Figure 1) that results in gross mitotic catastrophe, emergence of potentially immune-stimulatory micronuclei and synergistic cell death.

Our follow-up in vivo data demonstrates inhibition of tumor growth with DDK in combination with WEE1 inhibition that is improved with the addition of irinotecan. The frequency of DDK and WEE1 was reduced to once daily due to some evidence of weight loss with BID dosing and, while this mitigated toxicity, it also led to reduced efficacy (Figures 2-4). Unfortunately, trabectedin proved excessively toxic in the first mouse experiment and was not pursued further, although future combinations are of interest. The week 6 rechallenge in the second experiment appeared to indicate that the combination of DDK and WEE1 inhibition was needed, as the therapy overlapped for the first three days and then WEE1 inhibitor was given alone for Days 4-5. Intriguingly, only the triplet of the two novel inhibitors plus irinotecan had mice surviving past Week 6.

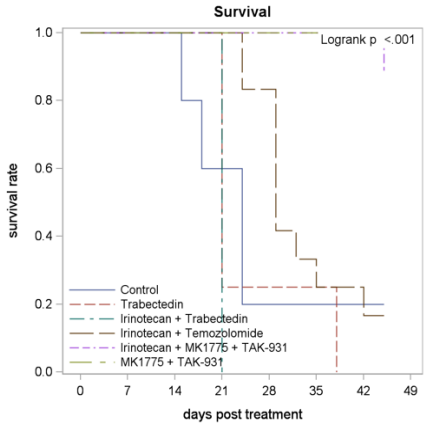
We are now planning experiments that will build upon these preliminary experiments but optimize the timing of DDK and WEE1 inhibition (alternating DDK and WEE1 inhibition). We will try treating the mice for longer than 3 weeks at a time to see if cure can be achieved. We are also looking to test the combination in additional cell lines, including a PDX model that is parafemoral.

Conclusion: The novel combination of two orally bioavailable inhibitors of DDK and WEE1 already separately in single-agent study in human patients, TAK-931 and MK1775, plus irinotecan may prove to be a promising combination to treat patients with Ewing sarcoma based on our preliminary data. To our knowledge, the combination of DDK and Wee1 inhibition has not been tested in humans to date but would be a logical next step.



1. Experiment 1

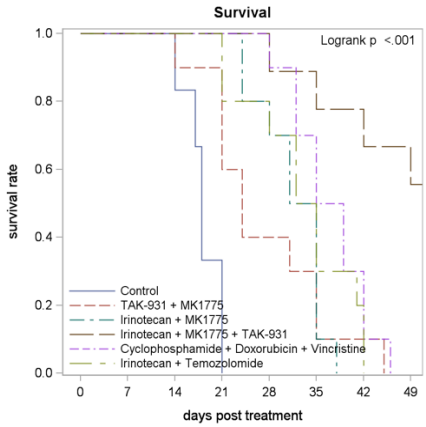
1.1 Survival



	Median (95% CI)
Total	35.0 (24.0, NR)
Control	24.0 (15.0, NR)
Irinotecan + MK1775 + TAK-931	NR (45.0, NR)
Irinotecan + Temozolomide	29.0 (24.0, 42.0)
Irinotecan + Trabectedin	21.0 (NR, NR)
MK1775 + TAK-931	NR (NR, NR)
Trabectedin	21.0 (21.0, 38.0)

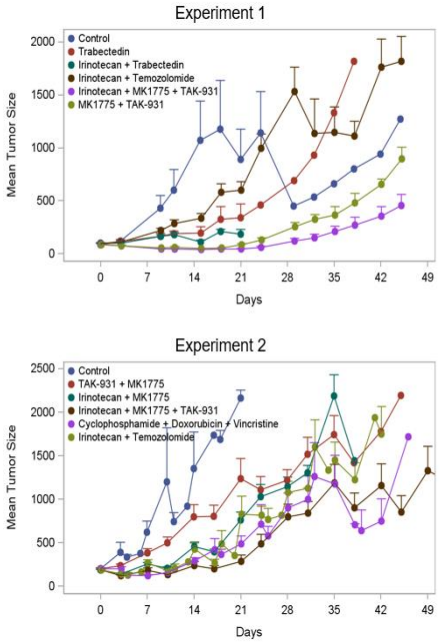
2. Experiment 2

2.1 Survival



	Median (95% CI)
Total	35.0 (28.0, 35.0)
Group 1	18.0 (14.0, 21.0)
Group 2	24.0 (14.0, 35.0)
Group 3	33.0 (24.0, 35.0)
Group 4	52.0 (28.0, 60.0)
Group 5	37.0 (28.0, 42.0)
Group 6	33.5 (21.0, 41.0)

3. Tumor Growth



(P 248) FIBROBLAST ACTIVATION PROTEIN ALPHA -DIRECTED IMAGING AND THERAPY OF SOLITARY FIBROUS TUMOR

Rainer Hamacher, MD[1]; Kim M. Pabst, MD[2]; Phyllis F. Cheung, PhD[3]; Christoph E. E. Heilig, MD[4]; Jennifer Hüllein, PhD[5]; Sven-Thorsten Liffers, PhD[6]; Sabrina Borchert, PhD[7]; Pedro Fragoso Costa, Msc, Dr[2]; Benedikt M. Schaarschmidt, MD[8]; Lukas Kessler, MD[2]; Monika A. Miera, n/a[1]; Margret Droste, PhD[3]; Merve Akbulut, n/a[1]; Johanna Falkenhorst, MD[1]; Fadi Zarrad, PhD[2]; Karina Kostbade, MD[1]; Ilektra A. Mavroeidi, MD[1]; Hanno Glimm, MD[9]; Lale Umutlu, MD[8]; Martin Schuler, MD[1]; Daniel Hübschmann, PhD[5]; Sebastian Bauer, MD[1]; Stefan Fröhling, MD[4]; Ken Herrmann, MD[2]; Jens T. Siveke, MD[3]; Hans-Ulrich Schildhaus, MD[7]; Wolfgang P. Fendler, MD[2], (1) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (2) Department of Nuclear Medicine, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (3) Bridge Institute of Experimental Tumor Therapy, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (4) Department of Translational Medical Oncology, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center (DKFZ), Heidelberg, Baden-Wurttemberg, Germany, (5) Computational Oncology, Molecular Precision Oncology Program, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Baden-Wurttemberg, Germany, (6) Bridge Institute of Experimental Tumor Therapy, West German Cancer Center, University Hospital Essen, Essen, Baden-Wurttemberg, Germany, (7) Institute of Pathology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (8) Department of Diagnostic and Interventional Radiology and Neuroradiology, West German Cancer Center, University Hospital Essen, Essen, Essen, Nordrhein-Westfalen, Germany, (9) Translational Medical Oncology, Faculty of Medicine and

University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Sachsen, Germany

Objective: Fibroblast activation protein alpha (FAP) is expressed at high levels in several tumors. Here, we report the expression pattern of FAP in solitary fibrous tumor (SFT) and its potential use as radiotheranostic target.

Methods: We analyzed FAP mRNA and protein expression in biopsies from SFT patients using immunohistochemistry and multiplex immunofluorescence. Tracer uptake and detection efficacy were assessed in patients undergoing 68Ga-FAPI-46 and 18F-FDG PET. 90Y-FAPI-46 radioligand therapy (RLT) was offered to eligible patients with progressive SFT.

Results: Among 813 patients and 126 tumor entities analyzed from the prospective observational MASTER trial of the German Cancer Consortium, SFT (n=34) had the highest median FAP mRNA expression. Protein expression was confirmed in tumor biopsies from 29 of 38 SFT patients (76%) in an independent cohort. Most cases showed intermediate to high FAP expression by immunohistochemistry (24 of 38 samples, 63%), which was primarily located on tumor cells. 19 patients who underwent 68Ga-FAPI-46 PET imaging demonstrated significantly higher tumor uptake with SUVmax 13.2 (IQR 10.2) and better mean detection efficacy of 94.5% (SEM 4.2%) as compared to 18F-FDG PET (SUVmax 3.2, IQR 3.1; 77.3%, SEM 5.5%). Eleven patients received a total of 34 cycles (median 3; IQR 2) 90Y-FAPI-46 RLT, resulting in disease control in nine patients (82%). Median progression free survival was 227 days (IQR 220).

Conclusion: FAP is highly expressed by SFT and may serve as target for imaging and therapy. Further studies are warranted to define the role of FAP-directed theranostic in the care of SFT patients.

(P 249) INVESTIGATING CLINICAL FACTORS ASSOCIATED WITH THE ESTABLISHMENT OF SUCCESSFUL PRIMARY HUMAN SOFT TISSUE SARCOMA CELL LINES

Victoria S. Coward[1]; Jen Dorsey*, PhD[2]; Nalan Gokgoz, PhD[1]; Yael Babichev, PhD[3]; Anthony Griffin, MSc[4]; Savtaj Brar, M.Sc., MD[4]; Peter Ferguson, MSc., MD[5]; Carol Swallow, MD, PhD[6]; Elizabeth Demicco, MD, PhD[7]; Brendan C. Dickson, MD[7]; Kim Tsoi, MD, PhD[5]; Jay Wunder, M.Sc, MD[5]; Irene Andrulis, PhD[8]; Rebecca Gladdy, MD, PhD[9], (1) The Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada, (2) Lunenfeld Tanenbaum Research Institute/Mount Sinai Hospital, Toronto, ON, Canada, (3) Sinai Healthcare System, Toronto, ON, Canada, (4) Mount Sinai Hospital, Toronto, ON, Canada, (5) University of Toronto, Toronto, ON, Canada, (6) Mount Sinai Hospital, Toronto, ON, Canada, Toronto, ON, Canada, (7) Sinai Health System, Toronto, ON, Canada, (8) The Lunenfeld-Tanenbaum Research Institute, Sinai Health System University of Toronto, Toronto, ON, Canada, (9) Mount Sinai Hospital, Sinai Health System, and Princess Margaret Cancer Centre Toronto, ON, Canada Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada University of Toronto, Toronto, ON, Toronto, ON, Canada

Objective: Soft tissue sarcomas (STS) are a heterogeneous group of neoplasms with over 70 distinct types, in aggregate accounting for 1% of adult cancers. Several of the most common adult STS include myxofibrosarcoma (MFS), undifferentiated pleomorphic sarcoma (UPS), and

leiomyosarcoma (LMS). All three represent pleomorphic malignancies with complex genomic copy number alterations, and poor survival rates, which, yet lack targetable molecular aberrations for therapeutic intervention. The study of STS and search for improved therapies is further complicated by the limited availability of well characterized, representative pre-clinical human STS models, therefore our program has endeavored to develop and characterize primary STS cell lines from resected patient specimens.

Methods: Tumor specimens (n=168) were collected from consented STS patients (n=166) undergoing biopsy or resection at Mount Sinai Hospital, Toronto. Specimens were immediately used to initiate cell lines or were cryopreserved for future use. Paired blood and/or normal tissue from each patient were stored in the biospecimen repository. To initiate cell cultures, tissues were physically dissociated into 1mm3 fragments. Fragments were plated in DMEM/F12 (UPS and MFS) or Opti-MEM (LMS) + 1% Penicillin / Streptomycin and 10-15% heat inactivated fetal calf serum (FBS) media with 2-10mg/ml collagenase. After 16-24 hours, digested fragments were dissociated by pipetting, washed and plated in media in a tissue culture-treated T75 flask. Media was changed after 7-10 days. Once the flask was 80% confluent, cells were passaged. We classified cell lines in to two categories based on growth in vitro: no or little growth (no adherent cells or cells that initially proliferated, but stopped before passage 6), and persistent growth (cells proliferated beyond passage 6). Select cases with bulk tumor, paired normal and cell line samples underwent Whole Exome Sequencing (WES). A Mutect2 analysis identified tumor-specific variations (TSVs) that were shared between the patient tumor and cell line but not in the blood normal control. Clinical information was collected for each patient. To determine if clinical characteristics were correlated with growth, Pearson chi squared tests and t tests were performed, while Kaplan Meier survival analysis and log rank tests were used to evaluate disease specific survival and overall survival. Significance was considered at $p < 0.05$.

Results: 168 specimens were collected from predominantly untreated, high-grade, primary tumors of the extremity (41.6%) (Table 1). In total, we successfully adapted 46 cell lines from 168 specimens (27%). 10 UPS cell lines were evaluated for TSV retention, and 6 cases retained TSVs beyond passage 6 (Table 2). 145 specimens used to initiate cell cultures were from primary tumors, and the remaining were derived from metastatic (n=18) or recurrent tumors (n=5). Success rates varied between the three types, where MFS had the highest rate of persistent growth from both primary and recurrent specimens (n=17/33, $p< 0.05$), followed by UPS (n=14/56) and LMS (n=15/79 LMS). When considering primary specimens only, successful cell line growth was more likely to occur from extremity tumors in patients who presented with metastatic disease, where the specimen was not treated before collection (Table 3, $p< 0.05$).

Conclusion: We have successfully established cell lines from MFS, UPS, and LMS specimens, albeit at different rates. Clinical associations may aid in prioritizing which tumors should be used to initiate cell cultures while TSV retention confirmed the cell lines that reflected the primary tumor. The cell lines and protocols established here have facilitated functional studies to better understand these entities on a molecular level and can assist in investigating potential therapies. Overall, the development of preclinical models of rare entities, such as MFS, UPS, and LMS, remains vital to improving the outcome for STS patients.

Patient and tumour characteristics table (n=166, 168)	
Characteristic	**Patients (n)**
Sex	
Male	76
Female	90
Age at Diagnosis	
Average (range)	62 (20-89)
IQR	55-72
55 or less	57
72 or more	45
Max. Diameter	
Average (range)	12.4 (1.2-32.8)
IQR,cm	5.5-14.5
5.5cm or less	32
14.5 cm or more	37
Presenting status of patient at time of specimen collection	
Primary (Local)	130
Primary (Recurrent)	5
Metastatic at time of procedure	33
Histology of collected specimen	
Leiomyosarcoma	79
Myxofibrosarcoma	33
Undifferentiated Pleomorphic Sarcoma	56
Treatment status of the collected specimen	
None	120
Systemic therapy	6
Radiotherapy	26
Systemic and Radiotherapy	16
Surgical Site (primary specimen)	
Extremity	81
Trunk	15
Abdomen & Retroperitoneum	52
Gynecological	3
Metastatic Site	17
Depth	
Extremity superficial	21
Extremity Deep	74
Abdomen & Retroperitoneum	73
Grade*	
1	13
2 or higher	127
Not Applicable	28
Disease Free Interval**	
Average (range)	28.5 (2-139)
*Not Applicable category includes 17 metastatic samples and 11 pre-treated samples that were not graded	
** Disease free interval of patient who presented with primary localized disease and later relapsed (n=130)	

Table 1. Characteristics of UPS, MFS, and LMS patients and specimens in the cell line study.

Cell line retained exonic variants by WES						
	IHRT5659	IHRT6665	IHRT4317	IHRT4248	IHRT4998	IHRT4606
Total bulk variants	38	39	298	279	66	24
Cell line retained variants	33	31	94	52	17	14

Table 2. Six UPS cell lines retained tumor-specific variants after extended time in culture. Variants were identified through a Mutect2 analysis of WES of the bulk primary tumor, paired patient blood, and cell line DNA, and were filtered for exonic SNVs or small indels with VAF over 0.1 in protein-coding genes.

Characteristics of primary specimen used to initiate cell lines (n=145)			
Characteristic		**Primary Specimen (% successful)**	**P Value**
Sex			
	Male	71 (24%)	0.03
	Female	74 (32%)	
Age at Diagnosis			
	Average (range)*	62 (20-89)	0.07
	IQR	53-72	
	53 or less	39 (23%)	0.87
	72 or more	42 (38.1%)	0.56
Max. Diameter			
	Average (range,cm)*	11.9 (1.8-38.5)	0.31
	IQR, cm	5.8-14.6	
	Less than 5.8cm	40 (32.5%)	
	More than 14.6cm	44 (22.7%)	0.69
Histology			
	Leiomyosarcoma	64 (17.2%)	
	Myxofibrosarcoma	31 (48.4%)	0.006
	Undifferentiated Pleomorphic Sarcoma	50 (28%)	
Presenting Status at time of collection			
	Primary M0	130 (23.8%)	0.003
	Primary M1 or Other	15 (60%)	
Treatment Status of the collected specimen			
	None	106 (35%)	
	Systemic therapy	2 (0%)	0.01
	Radiotherapy	25 (12%)	
	Systemic and Radiotherapy	12 (0%)	
Surgical Site			
	Extremity	80 (34%)	
	Trunk	14 (64.3%)	<0.001
	Abdomen & Retroperitoneum	50 (8%)	
	Gynecological	1 (0%)	
Depth			
	Extremity superficial	21 (47.6%)	
	Extremity Deep	77 (37.7%)	<0.001
	Abdomen & Retroperitoneum	47 (2%)	
Grade			
	1	13 (7.7%)	
	2 or higher	127 (31.5%)	0.07
	Not Reported	5 (0%)	
Two-sided Pearson Chi Square tests used unless otherwise specified, *One-sided T-Test with equal variances assumed			

Table 3. Associations between clinical characteristics and successful cell line growth in primary specimens. P values are univariate 2-sided Pearson Chi-square tests.

(P 250) SHARING HIGH-QUALITY SAMPLES AND DATA TO THE NF1 RESEARCH COMMUNITY: THE ROLE OF THE JOHNS HOPKINS NF1 BIOSPECIMEN REPOSITORY

Christine A. Pratilas, MD[1]; Jineta Banerjee, PhD[2]; Yang Lyu, PhD[3]; Ana Calizo, B.S.[1]; Kai M. Pollard, n/a[4]; Lindy Zhang, MD[5]; John M. Gross, MD[5]; Fausto Rodriguez, MD[6]; Robert J. Allaway, PhD[2]; Allan Belzberg, MD[1]; Angela Hirbe, MD,PHD[3]; Jaishri Blakeley, MD[1]; Stavriani Makri, Medical Doctor[7], (1) Johns Hopkins University, Baltimore, MD, (2) Sage Bionetworks, Seattle, WA, (3) Washington University in St. Louis, St. Louis, MO, (4) John Hopkins University, Reisterstown, MD, (5) Johns Hopkins Hospital, Baltimore, MD, (6) UCLA, Los Angeles, CA, (7) Johns Hopkins University School of Medicine, Baltimore, MD

Objective: Neurofibromatosis type 1 is a common neuro-genetic condition caused by mutations in the NF1 gene. It constitutes an autosomal dominant condition with a predisposition to benign and malignant tumors, including cutaneous neurofibromas (cNF), plexiform neurofibromas (pNF), atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP) and malignant peripheral nerve sheath tumors (MPNST). Progress in the development of nonsurgical therapy for pNF and MPNST has in the past been limited by several factors, including the limited access to primary tissue from patients with NF1 and the limited number of cell culture-based and animal models to adequately support research advances.

Methods: We have established and expanded the Johns Hopkins NF1 Biospecimen Repository, which includes quality-controlled, clinically and genomically- annotated samples from patients with NF1. Samples included in the biorepository are blood fractions, frozen tumor tissue, paraffin embedded tissue, single cell suspensions, and cell line and xenograft models to propagate primary human tissue and cells. Banked specimens also undergo comprehensive genomic characterization using whole exome sequencing (WES) and RNAseq, and data are

available through the NF Data Portal. A comprehensive clinically-annotated database includes NF1-associated clinical symptoms, tumor characteristics and outcomes data. An internal scientific review process allows for researchers outside our institution to request access to specimens, cell line and PDX models, and genomic data, and accompanying de-identified clinical information for their research.

Results: Since its inception in January 2016, over 300 unique samples have been banked (from 157 unique patients), and include plexiform neurofibroma (n=86), MPNST (n=56), cutaneous neurofibroma (n=89), blood fractions, and xenografts (n=7) specimens. 60 researchers from outside institutions have requested access to our specimens. RNAseq data (n=67) and WES data (n=102) are available through the NF Data Portal.

Conclusion: The Johns Hopkins NF1 Biospecimen Repository represents a high-quality, clinically, and genomically characterized resource for ongoing scientific efforts in the NF1 research community.

(P 251) EVALUATION OF POTENTIAL TARGETS FOR FLUORESCENCE-GUIDED SURGERY IN PEDIATRIC EWING SARCOMA: A PRECLINICAL PROOF-OF-CONCEPT STUDY

Zeger Rijs, MD[1]; Bernadette Jeremiasse, MD[2]; Laura Hiemcke-Jiwa, MD PhD[3]; Ella de Boed, MSc[3]; Peter Kuppen, N/A, PhD[1]; Cornelis Sier, PhD[1]; Pieter van Driel, MD PhD[4]; Michiel van de Sande, MD, PhD[1]; Marc Wijnen, MD PhD[3]; Alida van der Steeg, MD PhD[3], (1) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (2) Department of Surgery, Princess Maxima Center for Pediatric Oncology, The Netherlands;, Utrecht, Utrecht, Netherlands, (3) Princess Maxima Center for Pediatric Oncology, The Netherlands, Utrecht, Utrecht, Netherlands, (4) Isala hospital, Zwolle, Overijssel, Netherlands

Objective: Ewing sarcoma (ES) is an aggressive bone and soft-tissue tumor. Treatment generally consists of chemotherapy followed by surgery with- or without radiotherapy. Intraoperative distinction between healthy and tumorous tissue is challenging, especially after chemotherapy and at complex anatomical locations. Targeted fluorescence-guided surgery (FGS) can facilitate complete and less mutilating tumor resections by visualizing tumor tissue during surgery. This can be done with a tumor-specific target, a targeting moiety conjugated to a fluorophore (tracer), and a dedicated camera system. A previous systematic review selected CD99, CXCR4, NPY-R-Y1, LINGO-1, IGF-1R, and CD117 as promising targets for FGS of ES. Additionally, GD2 could be a promising target due to its known expression of 40-90% in ES biopsy samples and the clinical availability of a FGS tracer for neuroblastoma. As a crucial step towards developing targeted FGS for ES, this study aimed to evaluate the selected targets through an immunohistochemical analysis conducted on biopsies and resection specimens, including adjacent healthy tissue. Subsequently, three tracers with the highest potential for FGS were identified. Binding specificity of these tracers was assessed using cell line-based flow cytometry and immunofluorescence microscopy experiments. Finally, as proof-of-concept, a topical application experiment with the three selected tracers was performed on one freshly resected pediatric ES tumor and surrounding healthy tissue.

Methods: ES patients who gave informed consent for the use of their tissue at the time of surgery and underwent surgical resection between 2018 and 2022 were eligible for this study. Formalin-fixed paraffin-embedded (FFPE) biopsies (n=13) and corresponding whole tissue

sections of ES tissue samples with tumor and adjacent healthy tissue (n=8) were selected by a specialized sarcoma pathologist. Immunohistochemistry was performed to assess CD99, CXCR4, CD117, NPY-R-Y1, and IGF-1R expression in ES biopsies and resection specimens. Unfortunately, LINGO-1 and GD2 staining protocols did not work on FFPE tissue. Based expression and clinical availability of a targeted-tracer, anti-CD99, anti-CD117, and anti-GD-2 were further evaluated for binding specificity using flow cytometry and immunofluorescence microscopy. ES cell lines A673 and RD-ES were selected as lines to assess binding of the tracers, while neuroblastoma cell lines SK-N-BE and KCNR, and breast cancer organoid lines BC27T and BC62T served as positive or negative controls for the selected targets. Lastly, as proof of concept, anti-CD99, anti-CD117, and anti-GD2 tracers were topically applied to one freshly resected ES tumor and adjacent healthy tissue.

Results: Immunohistochemistry demonstrated moderate/strong CD99 and CD117 expression in ES-tumor samples, while adjacent healthy tissue had limited expression. Flow cytometry and immunofluorescence microscopy confirmed high CD99 expression, along with low/moderate CD117, and low GD2 expression in ES cell lines. Topical anti-CD99 and anti-GD2 application on ES-tumor showed fluorescence, while anti-CD117 did not show fluorescence for this patient.

Conclusion: Based on the immunohistochemical evaluation, preclinical in vitro experiments, and ex vivo topical application experiment on freshly resected Ewing sarcoma and adjacent healthy tissue, our findings suggest that CD99-targeting tracers hold promise for FGS of ES. CD117 and GD2 tracers could be potential alternatives. The next step towards development of ES-specific FGS tracers could be ex vivo topical application experiments on a large cohort of ES patients.

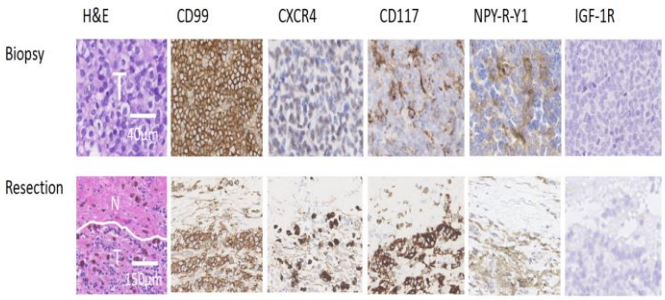
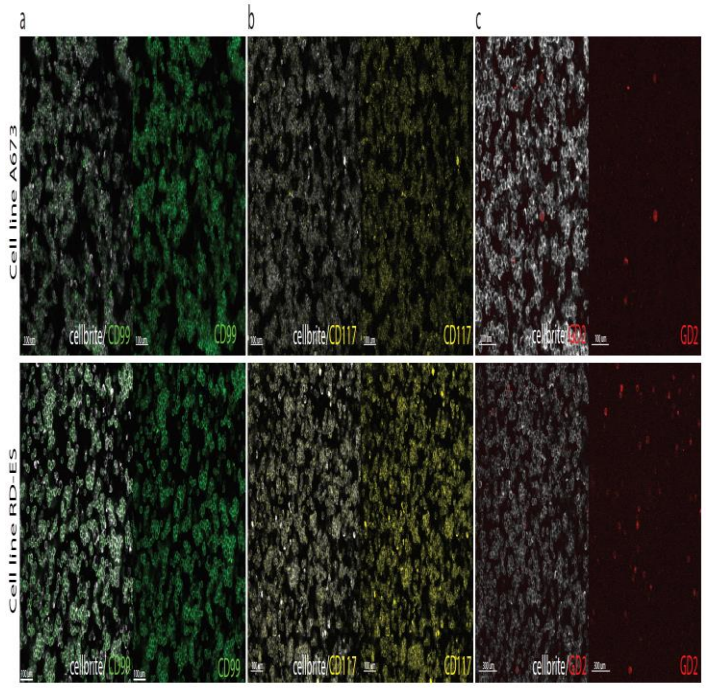
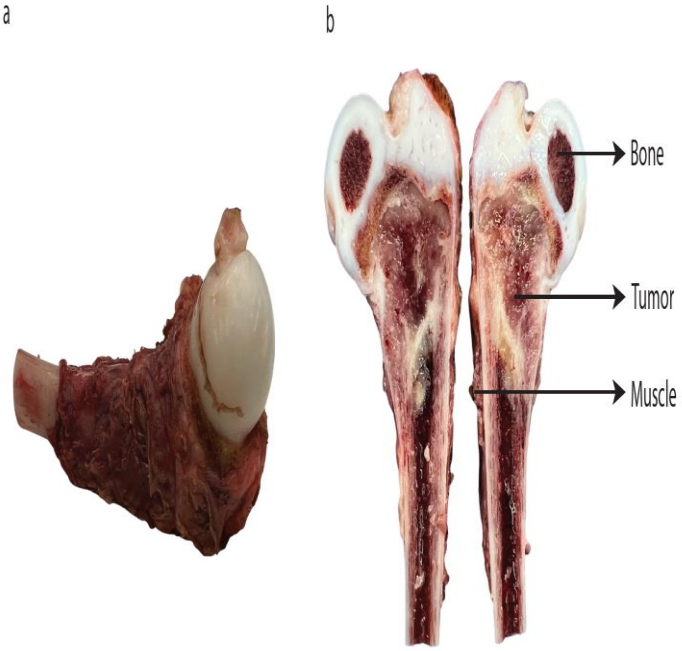
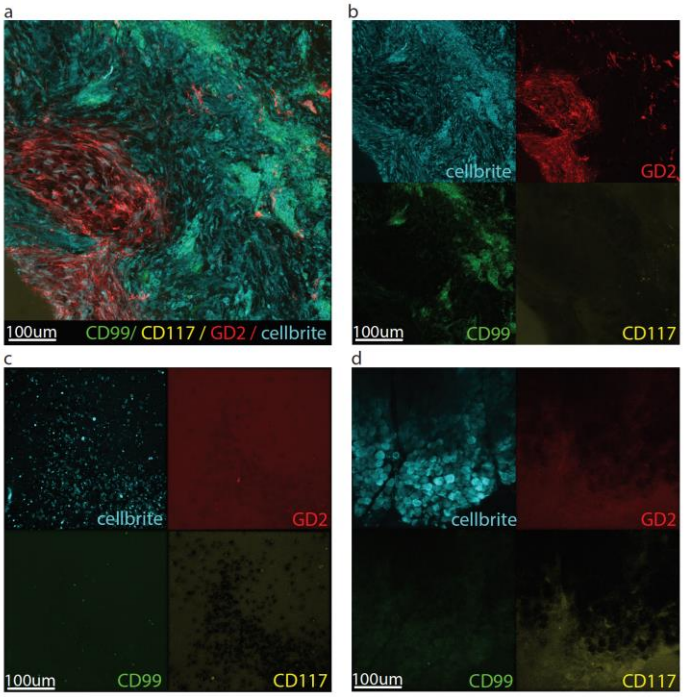


Figure 2. Representative IHC staining of biopsy and corresponding post-chemotherapy resection specimen from an ES patient. The H&E staining shows tumor cells in biopsy material (T), and adjacent healthy "normal" tissue (N) as well as tumor tissue (T) in resection material. Overall, CD99, CD117, and NPY-R-Y1 showed high percentages of stained ES cells in biopsy and resection material, with moderate to strong intensity, while staining in adjacent healthy tissue was limited. In this case CXCR4 displayed a moderate percentage of stained cells with mild to strong intensity, while IGF-1R did not show any stained cells.


a
b
c
Cell line A673
Cell line RD-ES
cellbrite/CD99
CD99
cellbrite/CD117
CD117
cellbrite/GD2
GD2


a
b
Bone
Tumor
Muscle



(P 252) STUDY OF NUTRACEUTICAL INTERVENTION WITH HIGH PHENOLIC EXTRA VIRGIN OLIVE OIL AND CURCUMIN FOR NEUROFIBROMATOSIS TYPE 1

Kyle B. Williams, PhD[1]; Helena Sverak, B.S.[2]; Robert T. Galvin, MD[2]; Pavlina Sverak, MD[2]; Christopher L. Moertel, MD[2]; David A. Largaespada, PhD[2], (1) Masonic Cancer Center University of Minnesota, Apple Valley, MN, (2) University of Minnesota, Minneapolis, MN

Objective: Neurofibromatosis Type 1 (NF1), a common genetic disorder caused by mutations in the tumor suppressor gene NF1, causes patients to develop Schwann cell tumors of the peripheral nervous system (neurofibromas). These can progress to malignant peripheral nerve sheath tumors (MPNSTs), a deadly soft tissue sarcoma. The development of MEK inhibitors for the treatment of plexiform neurofibromas has been a major milestone in the NF1 field, and they may be useful for other manifestations of the disease. However, many patients are unable to benefit from this therapeutic approach, possibly lacking a qualifying indication. Moreover, MEK inhibitors can have complex side effect profiles to manage, do not show efficacy in all patients, and the response is not durable if therapy is discontinued. There has been much interest in the NF1 patient community in exploring nutraceutical interventions which have been shown to mimic pharmaceutical inhibition of Ras and other NF1 relevant pathways including inflammation. These interventions could conceivably treat or prevent benign tumors in NF1 from developing or progressing.

Methods: Two nutraceutical compounds, oleocanthal and curcumin, have garnered particular attention. Oleocanthal is found in high phenolic extra virgin olive oil (HP-EVOO) and has been shown to have anti-inflammatory and anti-cancer properties. Curcumin is a bioactive compound found in turmeric and has been shown to have anti-inflammatory, antioxidant, and anti-cancer properties. Previously, a small clinical trial was conducted (Espositio et al, 2017), using dietary

intervention with high phenolic olive oil and curcumin, which showed promising results in NF1 patients. This study builds on the previous trial observations in the following ways: 1. We have an open and enrolling clinical trial to establish the safety and preliminary activity of a novel and more bioactive form of curcumin (SLCP Longvida®) and HP-EVOO supplementation in adult NF1 patients with cutaneous and plexiform neurofibromas (NCT05363267). The trial will establish plasma levels of curcumin and oleocanthal, monitor changes in inflammatory cytokines, utilize skin imaging to track cutaneous neurofibroma changes, and incorporate quality-of-life measurements. 2. We have examined signaling pathways and neurofibroma-relevant cell phenotypes impacted by curcumin and oleocanthal using human plexiform-like neurofibroma cells in vitro and animal models.

Results: In our pre-clinical work, we investigated the effect of curcumin, oleocanthal and their combination on signaling pathways in NF1-related cell lines. Our results show that the combination of the compounds at doses achievable in humans, but not single agents, produced potent inhibition of mTORC1 as evidenced by dramatically reduced pS6K, in NF1-deficient, immortalized human Schwann cells, comparable to the FDA approved mTOR inhibitor, everolimus. The combination treatment also inhibited activating phosphorylation of STAT3 and ERK in NF1-deficient Schwann cells. Strong inhibition of signaling pathways known to be important for neurofibroma formation by this combination prompted in vivo follow-up investigation. When used in an in vivo model of neurofibroma growth, promising results were again observed. Using NF1-deficeint immortalized human Schwann cells implanted into immunodeficient mice, control of tumor growth and an overall survival benefit was observed in animals receiving the oleocanthal/curcumin combination. Signaling pathway targets were also examined in treated tumor tissues vis immunohistochemistry.

Conclusion: The combination of oleocanthal and curcumin shows promise as a chemopreventive treatment for NF1 patients that could slow tumor growth and improve outcomes. We look forward to the results of our clinical trial.

(P 253) THE EFFICACY OF MEDICATED PATCHES IN SOFT TISSUE SARCOMAS: IN-VITRO AND EX-VIVO VALIDATION.

Laura Mercatali, PhD[1]; Alessandro De Vita, PhD[2]; Claudia Cocchi, PhD[2]; Enrico Lucarelli, M. Sc, PhD[3]; Chiara Bellotti, M. Sc.[3]; Ania Naila Guerrieri, M. Sc, PhD[3]; Chiara Liverani, PhD[4]; Giulia Sbanchi, n/a[4]; Maria Letizia Focarete, PhD[5]; Anna Liguori, n/a[5]; Luna Oliveti, MSc[5]; Chiara Gualandi, PhD[5]; Tommaso Frisoni, PhD[6]; Davide Maria M. Donati, MD[6]; Giacomo Miserocchi, MSc[4]; Silvia Vanni, PhD[7]; Chiara Spadazzi, PhD[7]; Chiara Calabrese, MSc[4]; Sofia Gabellone, n/a[7]; Federica Pieri, n/a[8]; roberto Casadei, MD[9]; Valentina Fausti, MD[10]; Lorena Gurrieri, MD[10]; Micaela Pannella, M.Sc, PhD[3]; Massimo Serra, MSc[3]; Claudia Hattinger, PhD[3]; Simona Paglia, PhD[3]; Toni Ibrahim, BSc, MSc, MD, PhD[3], (1) Istituto Ortopedico Rizzoli IRCCS, Osteoncology- Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, Bologna, Italy ; Istituto Scientifico Romagnolo per lo studio dei Tumori "Dino Amadori", Preclinic and Osteoncology Unit, Meldola, Italy, Bologna, Emilia-Romagna, Italy, (2) Istituto Scientifico Romagnolo per lo studio dei Tumori "Dino Amadori", Preclinic and Osteoncology Unit, Meldola, Italy, Meldola, Emilia-Romagna, Italy, (3) Istituto Ortopedico Rizzoli IRCCS, Osteoncology-Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, Bologna, Italy, Bologna,

Emilia-Romagna, Italy, (4) Istituto Romagnolo per lo studio dei Tumori "Dino Amadori" IRST IRCCS, Preclinic and Osteoncology Unit, Meldola, Italy, Bologna, Emilia-Romagna, Italy, (5) Department of Chemistry "Giacomo Ciamician", University of Bologna, 40126 Bologna, Italy, Bologna, Emilia-Romagna, Italy, (6) Istituto Ortopedico Rizzoli IOR IRCCS, SC Third Orthopaedic Clinic and Traumatology, Bologna Italy, Bologna, Emilia-Romagna, Italy, (7) Istituto Romagnolo per lo studio dei Tumori "Dino Amadori" IRST IRCCS, Preclinic and Osteoncology Unit, Meldola, Italy, Meldola, Emilia-Romagna, Italy, (8) Pathology Unit, "Morgagni-Pierantoni" Hospital, 47121 Forlì, Italy, Bologna, Emilia-Romagna, Italy, (9) Orthopedic Unit, Morgagni-Pierantoni Hospital, 47121 Forli, Italy, Forlì, Emilia-Romagna, Italy, (10) Clinical and Experimental Oncology, Immunotherapy, Rare Cancers and Biological Resource Center, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", 47014 Meldola, Italy, Meldola, Emilia-Romagna, Italy

Objective: Soft Tissue sarcomas (STS) are a heterogeneous group of rare tumors with a set of specific clinical challenges, such as tumor recurrence and wound complications. To improve patients' outcomes, innovative therapeutic strategies must be settled. Biomimetic materials with both, antitumor and regenerative agents could be used after surgery to induce the death of the residual cancer cells and restore the damaged tissue. To this purpose, we have developed a device designed for the treatment of patients with STS, and here we report its functional characterization.

Methods: The device was developed as a bioabsorbable, flexible and suturable patch. The patch consists of multiple layers of medicated materials, each with different functionality. The two outer layers consist of a biodegradable polymer (poly(L)lactic acid containing pluronic acid), while the inner layer is a methacrylate gelatin hydrogel. The anti-inflammatory drug Diclofenac (DICL) was added to both outer layers to ensure controlled release over 7 days, while the anticancer drug Epirubicin (EPI) was added to the inner hydrogel layer to obtain sustained release over 3 days. Drug release was assessed through HPLC-MS. The effect of single or combined (EPI-DCIL) drugs was evaluated in vitro and ex-vivo using commercial and patient-derived STS cell lines. Co-culture with normal fibroblasts was used to mimic soft tissue tumour microenvironment. Cell viability, growth, and necrosis were assessed. Immunofluorescence and confocal microscopy were also carried out to evaluate viability on STS cell-fibroblast co-culture.

Results: First, differential drug release from the devices was assessed. Results confirmed that EPI and DICL were released in a sustained manner over a period of 3 and 7 days, respectively. Using the epithelioid sarcoma cell line (VA-ES-BJ), it was confirmed that functionalized EPI had the same cytotoxic effect on both 2D-grown and patch-grown tumor cells. In the murine embryonic fibroblast cell line (3T3), DICL decreased fibroblast survival. Co-culture showed no further effects of DICL on tumor cells and confirmed the pharmacological effect of EPI. In ex-vivo analyses, only samples from patients diagnosed with myxofibrosarcoma were included in order to reduce variability due to different histotypes. In particular, primary cultures and a patient-derived myxofibrosarcoma cell line (MF-R3) were used. Both models showed a decrease in cell viability for EPI-medicated patches, although this was more evident in the patient-derived cell line, where a significantly reduced proliferation was also observed for DICL and EPI-DICL. LDH release, monitored as a sign of necrosis, was higher than unmedicated samples for DICL at day 3 and for EPI at day 7 in primary cultures. In the patient-derived cell line, a significant higher necrosis was observed at the end of the treatment for all the drug-medicated devices.

Conclusion: These results provide an in-vitro and ex-vivo proof of the efficacy of medicated patches in STS, establishing the basis for the application of these materials to control tumor growth. Further preclinical studies will be conducted to test whether they induce soft tissue

regeneration as well.

(P 254) A COLLECTION OF PATIENT-DERIVED XENOGRAFT MODELS OF GASTROINTESTINAL STROMAL TUMORS

Luna De Sutter, PhD student[1]; Agnieszka Wozniak, PhD[1]; Lore De Cock[2]; Chao-Chi Wang, PhD student[1]; Karo Wyns, Ba[1]; Kimberly Verbeeck, Master[3]; Ulla Vanleeuw, Ba[1]; Dirk Van Raemdonck, MD, PhD[4]; Baki Topal, MD, PhD[4]; Joris Jaekers, MD[4]; Daphne Hompes, MD, PhD[5]; Raf Sciot, MD, PhD[6]; Patrick Schöffski, MD, MPH[7], (1) Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Vlaams-Brabant, Belgium, (2) Department of General Medical Oncology, University Hospitals Leuven and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Oost-Vlaanderen, Belgium, (3) KU Leuven, Leuven, Vlaams-Brabant, Belgium, (4) UZ Leuven, Leuven, Vlaams-Brabant, Belgium, (5) University Hospitals Gasthuisberg Leuven, Leuven, Vlaams-Brabant, Belgium, (6) Department of Pathology, University Hospitals Leuven, Leuven Cancer Institute, Leuven, Leuven, Vlaams-Brabant, Belgium, (7) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium

Objective: Gastrointestinal stromal tumors (GIST) are the most common mesenchymal malignancy of the gastrointestinal tract. Most GIST harbor gain-of-function mutations in KIT or platelet-derived growth factor receptor alpha (PDGFRA) oncogenes, encoding for respective receptor kinases. Five tyrosine kinase inhibitors (TKI) are currently approved for treatment of advanced KIT- or PDGFRA-driven GIST. During TKI treatment most tumors develop secondary mutations in KIT or PDGFRA inducing drug resistance, so there is an unmet medical need for novel therapies. Patient-derived xenograft (PDX) models can be used for preclinical drug testing of new compounds in vivo. We recently expanded our established GIST PDX platform with new models with different molecular background.

Methods: Fresh tumor material was collected from consenting GIST patients who undergo surgery or diagnostic biopsy. A piece of the tumor tissue was transplanted subcutaneously on both flanks of partially immunodeficient, athymic NMRI nu/nu mice. Once tumor growth was observed, the tumor was re-transplanted to a next generation of mice (“passaging”). With every passage a piece of the tumor was collected; histopathological and molecular characteristics were compared with the characteristics from the original patient tumor.

Results: Since the launch of the XenoSarc platform in 2004 we have transplanted 149 tissue samples from 114 GIST patients, resulting in 12 established and well-characterized GIST models with different mutations and TKI sensitivity, and an additional 15 models in early stage (passage ≤2). All models have KIT or PDGFRA driver mutations. Three of these models harbor secondary mutations, of which two have a secondary mutation in KIT exon 17 and one in KIT exon 16. One model is characterized by an imatinib-resistant PDGFRA exon 18 p.D842V mutation. Drug testing in selected models already provided the preclinical rationale and supportive evidence for a number of ongoing and completed clinical GIST trials for some approved and experimental compounds.

Conclusion: We are expanding our established platform of well-characterized GIST PDX

models, that were proven to serve as an excellent tool for preclinical drug testing and tumor biology studies. The platform is available for cooperative projects with academic and commercial partners.

(P 255) EPIDEMIOLOGY OF NOVEL ACTIONABLE TARGETS IN SYNOVIAL SARCOMA: A TISSUE MICRO-ARRAY STUDY

Lore De Cock[1]; Flavia Paternostro, Student[2]; Ulla Vanleeuw, Ba[2]; Karo Wyns, Ba[2]; Che-Jui Lee, PhD[2]; Raf Sciot, MD,PhD[3]; Agnieszka Wozniak, PhD[2]; Patrick Schöffski, MD, MPH[4], (1) Department of General Medical Oncology, University Hospitals Leuven and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Oost-Vlaanderen, Belgium, (2) Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Vlaams-Brabant, Belgium, (3) Department of Pathology, KU Leuven and University Hospitals Leuven, Leuven Cancer Institute, Leuven, Vlaams-Brabant, Belgium, (4) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium

Objective: Synovial sarcoma (SySa) is one of the most common translocation-related soft tissue sarcomas. Patients with metastatic SySa have limited treatment options and a very poor prognosis. Several new experimental therapies are currently explored in clinical trials, including T cell-based therapies (NCT04044768, NCT01343043, NCT03462316) targeting cancer testis antigens such as New York esophageal squamous cell carcinoma 1 (NY-ESO-1) or melanoma-associated antigen 4 (MAGE-A4), and degraders targeting bromodomain containing BRD9 (NCT05355753). Preclinical studies investigate inhibitors of yes associated protein 1 (YAP1), transcriptional co-activator with PDZ-binding motif (TAZ) and inhibitors of C-X-C Motif Chemokine Receptor 4 (CXCR4). We explored the molecular epidemiology of these targets using a tissue micro-array (TMA) from clinical SySa samples and correlated these findings with corresponding clinicopathological data.

Methods: Expression of NY-ESO-1, MAGE-A4, YAP1, TAZ, CXCR4, and BRD9 was assessed in our SySa TMA by immunohistochemistry. This TMA consists of 302 cores from 101 clinical samples (2-3 cores/sample) from archival, paraffin-embedded material originating from 84 individual donors. The diagnosis of SySa was verified by a reference pathologist in the donor sample and is supported by the presence of the SSX-SS18 translocation in 89% of the cases (in 10% of the cases the analysis could not be performed and in 1% the fluorescence in situ hybridization (FISH) analysis was negative). Most samples (62%) were collected from the primary tumor, 22% and 16% originated from a metastatic lesion or local recurrence, respectively. Target expression was scored for each core as an H-score, defined as: (% stained cells with 0 intensity) × 0 + (% stained cells with 1+ intensity) × 1 + (% stained cells with 2+ intensity) × 2 + (% stained cells with 3+ intensity) × 3. For YAP1 and TAZ scoring was limited to nuclear expression, as this correlates with pathway activation. The average of the H-scores from multiple cores of the same donor was used for subsequent analyses. Only samples with at least 1% of positive cells (for NY-ESO-1 and MAGE-A4) or at least moderate staining (2+ in ≥30% or 3+ in ≥ 20% of the cells, for YAP1 and TAZ) in ≥1 core were considered positive. Mann-Whitney-U and Kruskal-Wallis tests were used for statistical analyses. Spearman's rank correlation coefficient was used to assess correlations. Cox proportional regression was used to correlate the H score with duration of overall survival.

Results: Expression of NY-ESO-1 and MAGE-A4 was found in 52% and 79% of SySa TMA samples, respectively. At least one of the cancer testis antigens was expressed in 83% of samples. Consistency of expression across cores from the same donor sample was observed in 88% (MAGE-A4) and 86% (NY-ESO-1) of samples. Nuclear expression of YAP1 and TAZ was observed in 88% and 64% of the samples, and in 61% of samples both proteins showed nuclear expression. Expression rates are comparable with previous reports in literature. There was no correlation between the expression levels of any of the target proteins and disease status at time of sampling, patient's survival, gender, patient's age and size or grade of the original tumor sample. The analysis of BRD9 and CXCR4 expression is currently ongoing.

Conclusion: MAGE-A4, NY-ESO-1, YAP1, and TAZ were expressed in a clinically relevant proportion of SySa cases represented in our TMA, which supports the utility of these antigens as targets for novel therapies in this disease. The consistency in the expression observed for MAGE-A4 and NY-ESO-1 suggests that the expression of these proteins can be reliably assessed in a biopsy. Although a prognostic role of these proteins has been described for other tumor types, we could not confirm this for SySa.

(P 256) EFFICACY AND TOXICITY OF VERTEPORFIN IN COMBINATION WITH PAZOPANIB TESTED IN A PATIENT-DERIVED SYNOVIAL SARCOMA XENOGRAFT

Lore De Cock[1]; Agnieszka Wozniak, PhD[2]; Ming Zhu, Sr., PhD[3]; Ulla Vanleeuw, Ba[2]; Raf Sciot, MD, PhD[4]; Guocan Wang, MD, PhD[3]; Hannah Beird, PhD[5]; Dejka M. Araujo, MD[6]; Patrick Schöffski, MD, MPH[7], (1) Department of General Medical Oncology, University Hospitals Leuven and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Oost-Vlaanderen, Belgium, (2) Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Vlaams-Brabant, Belgium, (3) Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA, Houston, TX, (4) Department of Pathology, University Hospitals Leuven, Leuven Cancer Institute, Leuven, Leuven, Vlaams-Brabant, Belgium, (5) Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA, Houston, TX, (6) The University of Texas MD Anderson Cancer Center, Houston, TX, (7) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium

Objective: Synovial sarcoma (SySa) is one of the more common soft-tissue sarcoma subtypes and is characterized by the presence of a pathognomonic translocation between chromosome X and 18, resulting in the formation of an SS18-SSX fusion oncogene. Patients with metastatic SySa have limited treatment options and a very poor prognosis. There is a high unmet medical need to develop more effective treatment options. Yes associated protein 1 (YAP1) is known to be overexpressed in the majority of SySa and is considered a potential therapeutic target in this disease. We performed preliminary in vitro experiments with the SySa celline (SYO-1) that expresses high levels of YAP1 and noticed that short hairpin antisense (shRNA) knockdown of YAP1 increased sensitivity to the angiogenesis inhibitor pazopanib, a drug with proven clinical activity in SySa. This led to an in vivo experiment, in which the YAP1 inhibitor verteporfin was combined with pazopanib in a patient-derived SySa xenograft (UZLX-STS7).

Methods: A 7-day exploratory toxicological study was performed in non-tumor bearing NMRI

nu/nu mice to evaluate potential drug-drug interactions and acute toxicity when combining pazopanib and verteporfin. Bodyweight and general well-being of mice was followed during the treatment period. Livers were collected for histopathological evaluation. For the efficacy study, NMRI nu/nu mice (n=29, 7-8 mice/group) were bilaterally transplanted with the xenograft model UZLX-STS7. Mice were treated for 23 days with either control, pazopanib (100 mg/kg once daily by oral gavaging), verteporfin (75 mg/kg every 2 days by intraperitoneal injection) or a combination of pazopanib and verteporfin (dose and schedule as for single agents). Efficacy assessment was based on the evaluation of the tumor volume, histopathology and immunohistochemistry.

Results: During the toxicological study, no acute toxicity was observed and no hepatotoxicity was seen on histopathological evaluation. The efficacy study showed that the combination of verteporfin and pazopanib led to tumor growth stabilization (tumor volume of 136% on the last day of treatment versus baseline) compared to any of the monotherapies (average relative tumor volume of 208% for verteporfin and 224% for pazopanib) or control (average relative tumor volume of 438%). However, the combination was poorly tolerated if mice were treated for a longer period, one mouse was found dead and one mouse needed to be sacrificed because of weight loss. Compared to tumors treated with control and verteporfin monotherapy, the combination treatment of verteporfin and pazopanib led to a relative decrease in mitotic and increase in apoptotic activity (similar as in tumors treated with pazopanib in monotherapy).

Conclusion: SySa is a tumor which may benefit from YAP1 inhibition. Combining the YAP1 inhibitor verteporfin with pazopanib led to stabilization of tumors in our in vivo experiment, but was poorly tolerated. Further investigations in this and other models are needed to evaluate whether dose adjustments or other YAP1 inhibitors might be less toxic and/or more effective.

(P 257) CHARACTERIZING THE EVOLUTION AND THERAPEUTIC RESPONSE OF UNDIFFERENTIATED PLEOMORPHIC SARCOMAS USING MOUSE MODELS

Jason E. Chan, MD PhD[1]; Jonathan Rub, BS[1]; Carleigh Sussman, BS[1]; William D. Tap, MD[1]; Cristina R. Antonescu, MD[1]; Samuel Singer, MD, FACS[1]; Doron Betel, PhD[2]; Tuomas Tammela, MD PhD[3], (1) Memorial Sloan Kettering Cancer Center, New York, NY, (2) Weill Cornell Medicine Graduate School of Medical Sciences, New York, NY, (3) MSKCC, New York, NY

Objective: Undifferentiated pleomorphic sarcomas (UPS) are aggressive sarcomas with few effective treatment options. Because of the rarity of these sarcomas and the paucity of representative disease models, little is known about how they develop and respond to therapeutics. The goal of our study is to identify cell state trajectories that define sarcomagenesis and to identify essential molecular features of UPS in response to chemotherapy.

Methods: We used single cell transcriptomics to study the initiation and evolution of UPS in an autochthonous genetically engineered mouse model. We profiled tumor development at multiple time points and computationally defined key cell state transitions during sarcomagenesis and during therapy response.

Results: Interestingly, we identify two discrete evolutionary pathways that arise in parallel in the mouse model. To determine how these discrete lineages evolve, we used barcoded lentiviruses

to initiate tumor development in the mouse model. By assessing the population of barcoded lentiviruses within the different transcriptional lineages, we determined there likely exists one or more cells of origin that define different UPS cell states. In addition, by perturbing these cell states with chemotherapy and comparing them to similar treatment in patient-derived xenografts, we uncovered core UPS cell states reflecting the response to chemotherapeutic pressure.

Conclusion: At least two major transcriptional subpopulations develop in parallel in a GEMM of UPS, with differing essential molecular features and potentially cells of origin. The intermediary cell states leading to these divergent transcriptomic populations may represent entry points for future therapeutic strategies. In addition, we identify cell states that enrich after chemotherapeutic in PDX models that mimic those that arise in the GEMM model, providing further insight into the human relevance of the GEMM model of UPS.

(P 258) THE CRITICALITY OF OXIDATIVE PHOSPHORYLATION IN SYNOVIAL SARCOMA METASTASIS

Megan Jagosky, MD[1]; Colin Anderson, MD[2]; Nury Steuerwald, PhD[1]; Jenny Chen, MS[1]; HsihTe Yang, PhD[1]; William Ahrens, MD[2]; Carol Farhangfar, PhD[1]; Joshua C. Patt, MD, MPH[3]; Malcolm H. Squires, MD[2]; Johann Hsu, MD[1]; Sharvil Desai, PhD[1]; Jeffrey S. Kneisl, MD FACS[2], (1) Levine Cancer Institute, Charlotte, NC, (2) Atrium Health, Charlotte, NC, (3) Atrium Health, Levine Cancer, Charlotte, NC

Objective: Synovial sarcoma (SS) is characterized by the pathognomonic translocation of the SS18 gene on chromosome 18 with the SSX gene on chromosome X. This change results in alteration of the SWItch/Sucrose Non-Fermentable (SWI/SNF) and polycomb complexes, which are ATP-dependent chromatin-remodeling complexes crucial in epigenetic changes and oncogenesis. Evaluating the resultant gene expression patterns from this translocation in SS is critical to determine what leads to increased risk of disease progression and metastasis.

Methods: The primary objective was to evaluate gene expression signatures in SS specimens to determine if there was a particular signal for increased risk of metastasis. Ribonucleic acid (RNA) sequencing was performed on primary tumor specimens from patients who developed metastasis and those who did not. Two analyses were performed, one including tumors that had and had not been exposed to prior treatment and another analysis with only treatment naïve tumors.
Gene set enrichment analysis (GSEA) was employed to evaluate which genes were over-represented, calculating an enrichment score (degree to which a gene set is overrepresented), normalized enrichment score (accounts for differences in gene set size and in correlations between gene sets and the expression dataset), family-wise error rate (FWER) p-value and nominal p-value. Given the relatively small number of patients, a nominal p-value of 0.10 was used for significance.

Results: Multiple pathways were enriched with genes over-expressed in the metastatic cohort in comparison to the non-metastatic cohort (Figure 1 and 2). Primary tumors that were eventually able to metastasize had increased expression of oxidative phosphorylation gene set (NES 1.55; p-val 0.078) and epithelial to mesenchymal transition gene set (NES 1.46; p-val 0.036) (table 1). Treatment naïve tumors showed an even wider array of gene set expression (table 2) and the oxidative phosphorylation gene set continued to be significantly represented

(NES 1.6, p-val 0.067). Within the oxidative phosphorylation gene set, translocase of outer mitochondria membrane 22 (TOMM22), mitochondrial ribosomal protein (MRPL11), voltage dependent anion channel (VDAC2), peptidase mitochondrial processing subunit alpha (PMPCA), aconitase 2 (ACO2) were all highly enriched (running ES < 0.05).
Multiple pathways were enriched with genes over-expressed in the metastatic cohort in comparison to the non-metastatic cohort (Figure 1 and 2). Primary tumors that were eventually able to metastasize had increased expression of oxidative phosphorylation gene set (NES 1.55; p-val 0.078) and epithelial to mesenchymal transition gene set (NES 1.46; p-val 0.036) (table 1). Treatment naïve tumors showed an even wider array of gene set expression (table 2) and the oxidative phosphorylation gene set continued to be significantly represented (NES 1.6, p-val 0.067). Within the oxidative phosphorylation gene set, translocase of outer mitochondria membrane 22 (TOMM22), mitochondrial ribosomal protein (MRPL11), voltage dependent anion channel (VDAC2), peptidase mitochondrial processing subunit alpha (PMPCA), aconitase 2 (ACO2) were all highly enriched (running ES < 0.05).

Conclusion: There are two main metabolic pathways for cancer metabolism, glycolysis (generating 2 molecules of ATP) and oxidative phosphorylation (generating 36 molecules of ATP). Even in the presence of sufficient oxygen, most cancers use glycolysis preferentially. While a minority of cancers rely on mitochondrial metabolism, the center of which is oxidative phosphorylation, our findings suggest that SS may be one of those cancer types. Additionally, other tumors with mutant SWI/SNF complexes, including some lung cancers, have demonstrated reliance on oxidative phosphorylation and demonstrated preclinical responses to oxidative phosphorylation blockers. Mitochondrial metabolism can be inhibited by numerous agents, some as commonplace as metformin and statins. There is retrospective data that patients with soft tissue sarcoma that take metformin have improved survival, even after controlling for many confounding factors. Targeting the oxidative pathway is possibly a novel option for treatment of SS and should be investigated.

Table 1. GSEA of primary tumors that metastasized (all specimens)

Gene set	Nominal p-value	FWER p-val	NES
Oxidative phosphorylation	**0.078**	**0.370**	**1.55**
Epithelial mesenchymal transition	0.036	0.479	1.46

Table 2. GSEA of primary tumors that metastasized (treatment naïve)

Gene set	Nominal p-value	FWER p-val	NES
Allograft rejection	0.000	0.011	1.99
Coagulation	0.008	0.100	1.79
Epithelial mesenchymal transition	0.025	0.177	1.71
Complement	0.012	0.177	1.71
TNFA signaling	0.031	0.201	1.68
MYC targets	0.048	0.226	1.65
ROS pathway	0.045	0.255	1.63
Oxidative phosphorylation	**0.067**	**0.296**	**1.60**
Myogenesis	0.011	0.298	1.60

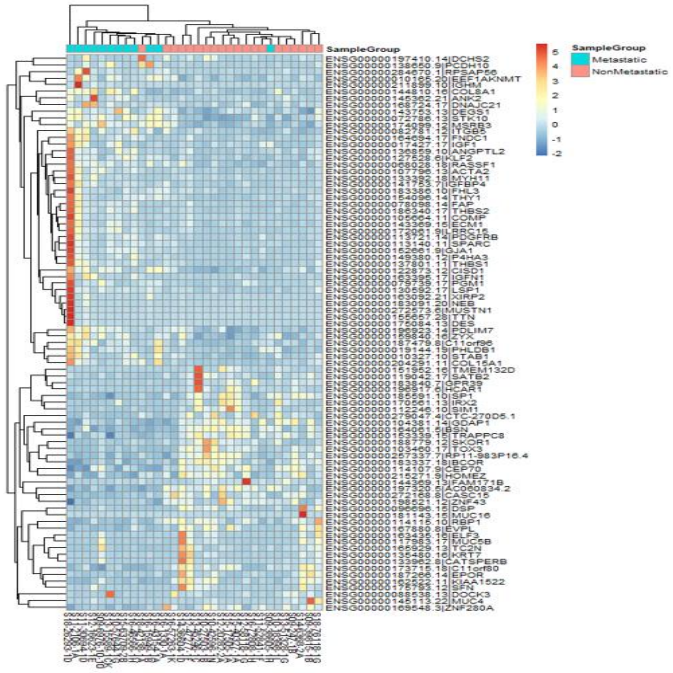


Figure 1. RNA sequencing heat map of all analyzed SS specimens

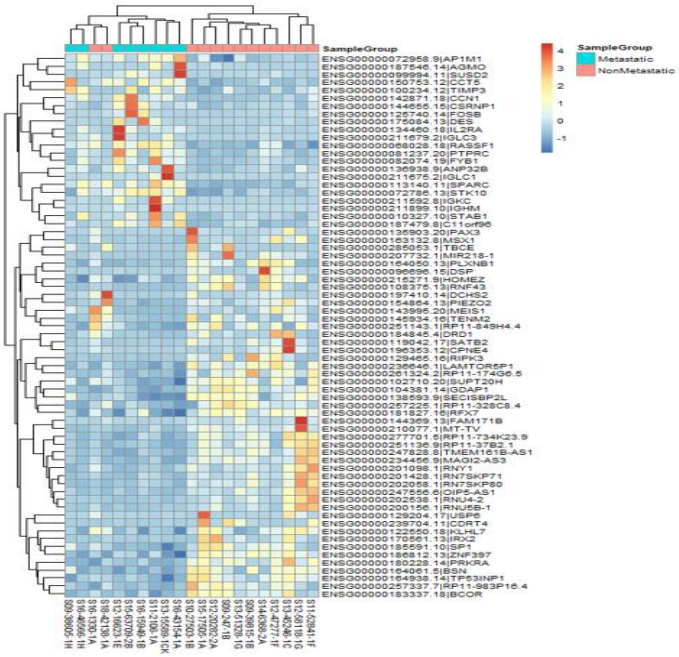


Figure 2. RNA sequencing heat map of treatment naïve SS specimens

(P 259) LOVASTATIN INDUCES NOVEL CELL DEATH THROUGH THE IMPAIRED MITOCHONDRIA IRON UTILIZATION IN OSTEOASRCOMA CELLS.

Kanya Honoki, MD, PhD[1]; Shingo Kishi, N/A, MD, PhD[2]; Shiori Mori, N/A, PhD[3]; Shinji Tsukamoto, N/A, MD, PhD[3]; Hiromasa Fujii, MD, PhD[3]; Yasuhito Tanaka, N/A, MD, PhD[3]; Hiroki Kuniyasu, N/A, MD, PhD[3], (1) Nara Medical University, Kashiara, Nara, Japan, (2) Nozaki Tokushukai Hospital, Daito, Osaka, Japan, (3) Nara Medical University, Kashihara, Nara, Japan

Objective: It has been reported that the mechanism of tumor-specific cell death caused by metabolic regulation has been different from normal cells. In this study, we report the effect of lovastatin, a fat-soluble statin, which we have found from screening of 400 agents, that specifically cause mitochondrial damage leading the cell death in human osteosarcoma cell lines.

Methods: The action of lovastatin was investigated using human osteosarcoma cells SaOS2, U2OS, MG63 and MSC. Cell viability was evaluated by MTS assay. Mitochondria divalent iron was evaluated by Mito-Ferro Green staining. Mitochondria function was evaluated by TMRE staining. Mitochondria ROS were observed as super oxide, H2O2, and hydroxyl radical, respectively, with fluorescent dyes. Mitophagy and iron metabolism-related proteins were evaluated by RT-PCR and western blot.

Results: IC50 was 10-15μM in osteosarcoma cells compared to 30μM in MSCs. Iron chelator, deferoxamine (DFO) increased cell death among various cell death inhibitors under 10μM lovastatin treatment (Figure 1), and this cell death was rescued by iron sulfide administration (Figure 2). Lovastatin administration reduced mitochondria membrane potential and decreased mitochondrial volumes, but increased superoxide, •OH and mitophagy, and all of which were also rescued by iron administration. In addition, the expression levels of transferrin and DMT1 increased with lovastatin administration, and excessive accumulation of iron and divalent iron, and a relative decrease of trivalent iron were observed in mitochondria. Since statin inhibits HMG-CoA reductase, administration of mevalonate, downstream of HMG-CoA, also rescued cell death and mitochondrial impairment as well as iron administration.

Conclusion: Although the induction of ferreoptosis by statin has been reported previously, the current results from our study suggest that cell death enhanced in osteosarcoma cells by DFO under lovastatin treatment could be a different effect from the known cell death pathway through the impaired conversion of iron from divalent to trivalent in mitochondria, since if ferreoptosis is the cause of the cell death, DFO should rescue it. In addition, CoQ deficiency is supposed to increase the superoxide that rapidly converts trivalent iron to divalent iron, and from these results, it is considered that lovastatin causes iron utilization disorder mediated by CoQ synthesis impairment, and induces cell death of a novel mechanism specific to osteosarcoma.

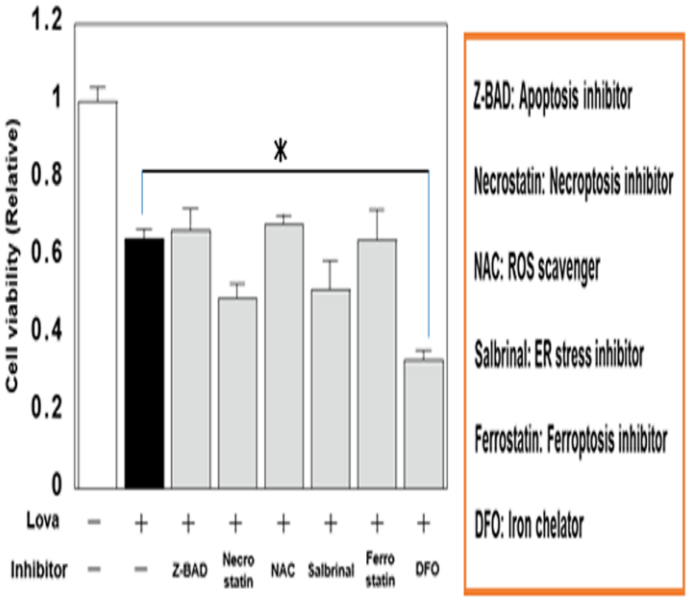


Figure 1

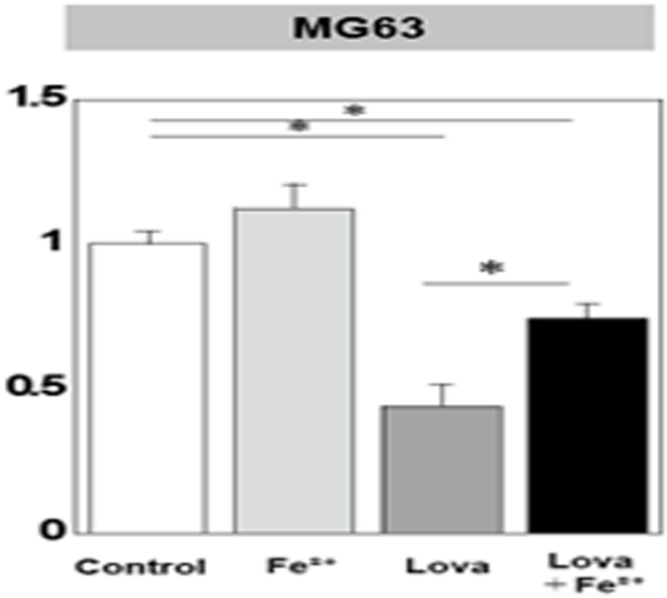


Figure 2

(P 260) TARGETING HOMOLOGOUS RECOMBINATION DEFICIENCY (HRD) IN LEIOMYOSARCOMA WITH COMBINATION OF PARP INHIBITORS (PARPI) AND CHEMOTHERAPY (CTX).

Olga Vornicova, MD[1]; Joshua Nash[2]; Adam Shlien, PhD, FCCMG[2]; Richard Marcellus, PhD[3]; Rima Al-Awar, N/A, PhD[3]; Albiruni Ryan Abdul Razak, MB BCh, BM BCh[4]; Rebecca Gladdy, MD, PhD[5]; Yael Babichev, PhD[6], (1) BC Cancer Victoria, Victoria, BC, Canada, (2) The Hospital for Sick Children, Toronto, ON, Canada, (3) OICR, Toronto, ON, Canada, (4) Princess Margaret Cancer Center, Toronto, ON, Canada, (5) Mount Sinai Hospital, Sinai Health System, and Princess Margaret Cancer Centre Toronto, ON, Canada Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada University of Toronto, Toronto, ON, Toronto, ON, Canada, (6) Sinai Healthcare System, Toronto, ON, Canada

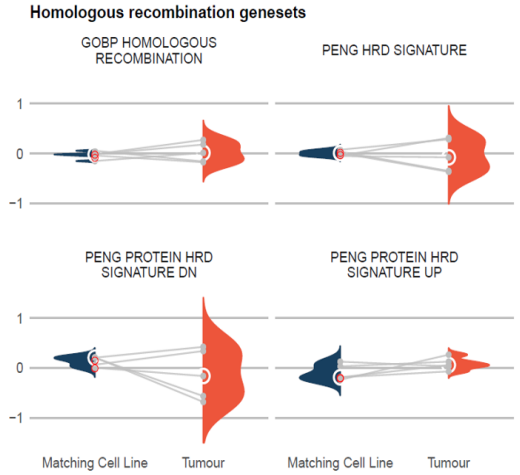
Objective: Leiomyosarcomas (LMS) are genetically heterogeneous tumors that arise from smooth muscle. Currently, the mainstay of systemic treatment for patients with advanced/metastatic disease is doxorubicin (Dox) based CTx. Dox causes double strand (DSB) DNA breaks in exposed cells. Several genomic analyses of LMS reveal defects in the homologous recombination (HR) DNA repair pathway in about half of patients, consistent with a druggable “BRCAness” phenotype. In HR deficient cells, DSB are restored by non-homologous end joining (NHEJ), which can be impaired by PARPi. Therefore, the combination of chemotherapy and PARPi may represent a promising therapeutic option for LMS. To validate this hypothesis, we performed a series of experiments in vitro on LMS cell lines. To confirm that HR deficiency mechanisms were present in the experimental cell lines, we performed a comparative transcriptome analysis of tumor/cell line paired samples.

Methods: Dox, Docetaxel (Doc) and Temozolomide (Tmz) were evaluated in combination with several PARPi (Olaparib [Ola], Niraparib [Nira] and Talazoparib [Tala]) at 12 different drug concentrations. All drug concentrations were chosen according to EC50, identified in single drug experiments on 12 LMS cell lines. Drug combinations were tested in a high throughput manner on four LMS cell lines of either gynecological (GY), abdominal (A) or extremity (E) origin. Cells were incubated with each combination for 7 days. Evaluation of drug combination effect was performed using a Bliss synergy score. This system quantifies the degree of synergy as multiplicative effect of single drugs as if they acted independently. Specifically, with a synergy score of -5 to 5, the interaction between two drugs is considered as additive: <-5 antagonistic and > 5 synergistic, and therefore a promising combination.
Tumor-cell line pairs were then selected for transcriptome analysis. All analyses were performed in R (v4.2.3). Cell line and tumour data were combined and then normalized using edgeR (v3.40.2) calcNormalFactors function. Protein coding genes were used (n = 18471). The normalized expression data were then run through GSVA (v1.46.0), using all available genesets from the Molecular Signature Database. GSVA scores were then analyzed using limma (v3.54.2) with cell lines vs. tumour as the coefficient of interest while accounting for cell line/tumour pairing. Benjamin Hochberg adjusted p values were reported.

Results: Anticancer activity, ranging from additive to synergistic was seen with all combinations. Results were consistent among all cell lines, independent of site of cell line origin (Table 1). The most synergistic combination in the majority of LMS cell lines was Dox or Tmz when combined with Tala, reaching up to 15 % and 27% above Bliss respectively. In contrast, Doc only had an additive effect with all analyzed PARPi. Comprehensive transcriptome analysis revealed similar expression of gene sets involved in HR between cell lines and corresponding tumors (Fig1, Table 2).

Conclusion: These data suggest that combining Dox or Tmz with PARPi may be a promising treatment option for LMS patients. Transcriptome analysis confirmed the presence of similar HRD mechanisms between tumors and corresponding cell lines. Recent clinical studies support this notion in uterine LMS. Importantly, our data suggest that such an approach may be extended to all sites of LMS. Preclinical in vivo studies are underway to identify the most promising combinations for future clinical trial design

Figure 1. HR gene sets expression in paired samples: LMS cell line vs parental tumor.



Legend: Semi-violin plots representing expression of HR genesets in LMS cell lines (navy blue) and corelating parental tumors (orange). These include two A LMS (STS 210, STS 551), four E LMS (STS 137, STS 714, STS 738, STS 652), and two GY LMS (STS 739, STS 685). Grey lines are connecting paired samples, red dots represent gene expression in the cell lines where tumor was not available for sequencing (STS 137, STS 551).

Table 1. Value of synergy of CTx and PARPi in analyzed drug combinations

	Dox (0.0013-2.5 μM) *			Tmz (0.1-40 μM) *			Doc (0.02-50 nM) *		
0.026-10 μM*	Tala	Ola	Nira	Tala	Ola	Nira	Tala	Ola	Nira
STS 39	5-12%	2-4%	4-6%	10-20%	2-10%	2-7%	3-6%	4-6%	-10-0%
STS 137	10-15%	4-8%	4-7%	2-8%	7-14%	5-13%	-10-0%	2-4%	2-4%
STS210	5-8%	3-7%	2-5%	9-27%	5-15%	-10-5%	6-11%	-10-0%	3-5%
SKLMS1	-5-0%	2-6%	4-7%	-10-0%	10-13%	6-8%	-10-0%	-10-0%	2-4%

Legend: Evaluation of synergy of CTx and PARPi in LMS cell lines (STS 39-GY, STS 137-E, STS 210- A, SKLMS1- GY). BLISS calculation for combination therapy is depicted by colour – synergy, additive, or antagonistic effect. Drug range represented in values, used for all 12 concentrations.

Table 2. Comparative analysis of gene sets, involved in DNA repair between LMS parental tumors and correlating cell lines.

Pathway	Gene set	logFC Tumours vs Cell Lines	p-value	Source/ publication
Homolog-ous recombi-nation	GOBP_HOMOLOGOUS_RECOMBINATION	0.06	0.62	http://amigo.geneontology.org/amigo/term/GO:0035825
	PENG_PROTEIN_HRD_SIGNATURE_DN	-0.27	0.18	PMID: 24553445
	PENG_PROTEIN_HRD_SIGNATURE_UP	0.17	0.23	
	PENG_HRD_SIGNATURE	-0.04	0.73	
PARP	MCGRAIL_PARPi_SENSITIVE_DN	-0.31	0.03	PMID: 28649435
	MCGRAIL_PARPi_SENSITIVE_UP	0.12	0.36	

(P 261) EFFICACY OF BET INHIBITORS IN SOLITARY FIBROUS TUMOR: AN IN VITRO EVALUATION

Jose L. Mondaza-Hernandez, Master degree[1]; Yi Li, Yi Li[2]; Pablo Romero, Master degree[1]; Marta Martín-Ruiz, PhD[3]; john Tran Nguyen, Master Degree[2]; David S. Moura, PhD[1]; Heather Naomi Hayenga, PhD[2]; Leonidas Bleris, PhD[2]; Javier Martin-Broto, MD, PhD[4], (1) Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM),, Madrid, Madrid, Spain, (2) Department of Bioengineering, University of Texas at Dallas, Richardson, TX, (3) Instituto de Investigaciones Sanitarias Fundación Jiménez Díaz, Madrid, Madrid, Spain, (4) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain

Objective: Solitary fibrous tumors (SFTs) are rare mesenchymal neoplasms driven by NAB2-STAT6 gene fusion. Currently, the therapeutic options available for advanced unresectable SFTs are limited to antiangiogenics, with cycles alternating different molecules. However, outcomes are still insufficient, with mainly temporal partial responses achieved. Thus, there is an urgent need for novel therapeutic approaches. Bromodomain and extra-terminal (BET) protein inhibitors have shown promising results in several types of cancers, including rhabdomyosarcoma, by targeting the transcription of genes regulated by BET proteins. We explore the potential effectiveness of BET inhibitors in SFT, as the role of targeted therapies in this entity has yet to be determined.

Methods: Three different BET inhibitors, Mivebresib, Pelabresib, and ABBV-744, were evaluated to determine their antitumor activity in SFT cell lines. The SFT models used in this study were INT-SFT (large-T antigen transformed) and IEC139 (primary). As non-translocated controls, we employed leiomyosarcoma cell lines, specifically CP0024 (primary) and SK-UT-1

(commercial). To assess the effects of BET inhibitors on SFT cell viability and death, different assays were conducted: cell viability assays using MTS colorimetry, flow cytometry apoptosis assays, and Western blot (WB) analysis.

Results: Our findings revealed that BET inhibitors reduced cell viability in SFT cell lines to a greater extent than in leiomyosarcoma, particularly Mivebresib (IC50 of 5.17x10-10, 1.91x10-9 vs 1.24x10-7, 2.86x10-8). Specificity for SFT was confirmed by flow cytometry apoptosis assays and increased expression of apoptosis markers (cleaved PARP-1) by WB. WB analysis demonstrated upregulation of γ-H2AX, a DNA-double strand break marker, and downregulation of RAD51, a protein involved in homologous recombination, suggesting a potential DNA damage-driven mechanism of action for BET inhibitors in SFTs. In addition, a synergistic effect was observed between BET inhibitors and Rucaparib, a commonly used PARP inhibitor (Combination Index of 0.42 and 0.27 for INT-SFT and IEC139 respectively).

Conclusion: BET inhibitors demonstrated efficacy and specificity for SFT in vitro models, potentially through targeting the HR pathway. Further investigations, including in vivo experiments, are necessary to elucidate the underlying mechanisms and evaluate clinical efficacy. The knowledge gained from these studies can lay the groundwork for potential clinical trials that will enable us to assess their safety, efficacy, and potential side effects in the context of SFT.

(P 262) ESTABLISHMENT AND CHARACTERIZATION OF THE FIRST RADIATION-INDUCED, PATIENT-DERIVED ANGIOSARCOMA CELL LINE

Lorenzo D'Ambrosio, MD[1]; Alessandra Merlini, MD, PhD[2]; Silvia Brusco[3]; Martina Olivero, PhD[3]; Gabriella Doronzo, PhD[3]; Luisella Righi, MD, PhD[3]; Francesca Napoli, PhD[4]; Angela Listì, PhD[3]; Marco Volante, MD[3]; Ida Rapa, PhD[3]; Giorgia Giordano, BSc[5]; Claudio Isella, PhD[5]; Federica Santoro, MD[6]; Sandra Aliberti, MD[5]; Valeria Pavese, MD[3]; Martina Rabino, MD[3]; Giorgio Scagliotti, MD[3]; Giovanni Grignani, MD[7]; Ymera Pignochino, PhD[5], (1) University if Turin, Turin, Italy, Torino, Piemonte, Italy, (2) Department of Oncology, University of Turin, Torino, Piemonte, Italy, (3) Department of Oncology, University of Turin, Turin, Piemonte, Italy, (4) Department of Oncology, University of Turin, Orbassano, Piemonte, Italy, (5) Candiolo Cancer Institute IRCCS FPO, Turin, Piemonte, Italy, (6) Città della Salute e della Scienza di Torino, Turin, Piemonte, Italy, (7) Candiolo Cancer Institute, FPO – IRCCS, Turin, Piemonte, Italy

Objective: Radiation-induced breast angiosarcoma (RIBAS) represents an unmet medical need, characterized by high recurrence rate, limited therapeutic options and dismal prognosis in advanced/metastatic stages. To the best of our knowledge, angiosarcoma preclinical models available worldwide are limited to five UV-induced cutaneous angiosarcoma cell lines, and one patient-derived xenograft model for radiation-induced angiosarcoma. Our objective was to develop a bona fide RIBAS cell line (RIAS001), describing its immunophenotypic features, tumorigenicity in mice, and RNA expression profile.

Methods: RIBAS cells were harvested from pleural effusion of a patient affected by metastatic RIBAS, cultured (named as RIAS001 cell line), and implanted subcutaneously into the right flank of 7-week old NOD-SCID IL2R γnull mice (cell-line derived xenograft; CDX). Total RNA was extracted from cell lines and snap-frozen tumor tissues; total RNA Sequencing was

performed by Illumina TruSeq Stranded Total RNA gold Kit and NovaSeq6000 System. RIAS001 cells were characterized by immunohistochemistry, immunofluorescence, transcriptomic profile.

Results: We successfully isolated RIBAS cells from the pleural effusion of a 63-year old woman affected by secondary, radiation-induced angiosarcoma of the breast. We cultured cells in vitro, establishing doubling time and optimizing cell culture conditions with different culture media and growth factors.
In detail, we tested M199 (HUVEC cell culture medium), DMEM KnockOut (optimal for primary patient-derived cell lines), and DMEM High Glucose, which proved as the most suited for RIAS001 growth. From the cultured cells (RIAS001), a xenograft mouse model (2 generations) was established, and its morphological and immunohistochemical features were compared to the patient tumor sample as shown in Figure 1. Both the cell line and the CDX retained immunophenotypic angiosarcoma features, as shown by immunofluorescence at confocal microscopy (Figure 2) and immunohistochemistry (Figure 1). The RNA expression profile of RIAS001 and RIAS001-CDX was analyzed in comparison to HUVEC cells, and to other cell lines and CDX derived from non-angiosarcoma STS. The analysis confirmed high expression of CD31, ERG, and MYC in RIAS001/RIAS001-CDX.

Conclusion: We established the first bona fide RIBAS cell line described so far (RIAS001). We have shown tumorigenicity in mice, and retained morphological and immunophenotypic features of RIAS001/RIAS001-CDX compared to the patient's tumor samples. Therefore, RIAS001 represents a valuable and versatile preclinical radiation-induced angiosarcoma model, which could foster future angiosarcoma research.

Figure 1.

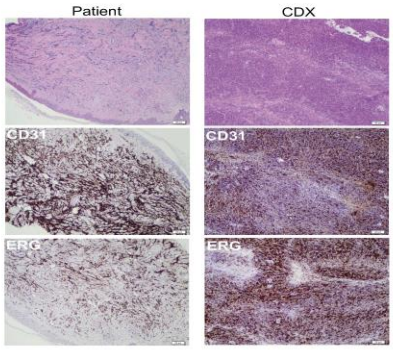
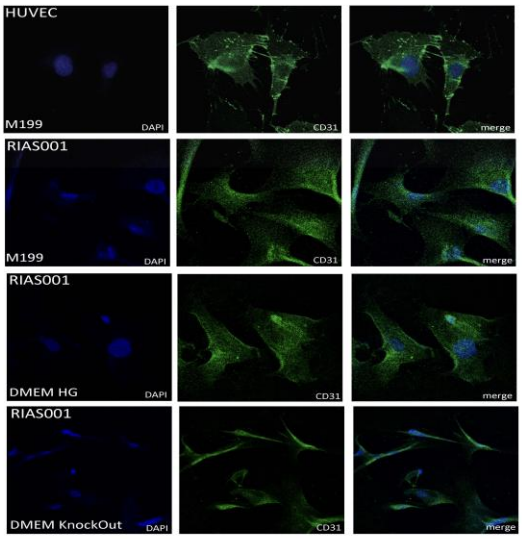


Figure 2.



(P 263) **HER2 AS A POTENTIAL NOVEL THERAPEUTIC TARGET IN SOFT TISSUE SARCOMAS**

Bruno Vincenzi, MD, PhD[1]; Francesco Pantano, MD, PhD[1]; Michele Iuliani, PhD[2]; Sonia Simonetti, PhD[2]; Silvia Cavaliere, PhD student[2]; Alessandro Mazzocca, MD[1]; Giuseppe Tonini, MD, PhD[1], (1) Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Lazio, Italy, (2) Università Campus Bio-Medico di Roma, Rome, Lazio, Italy

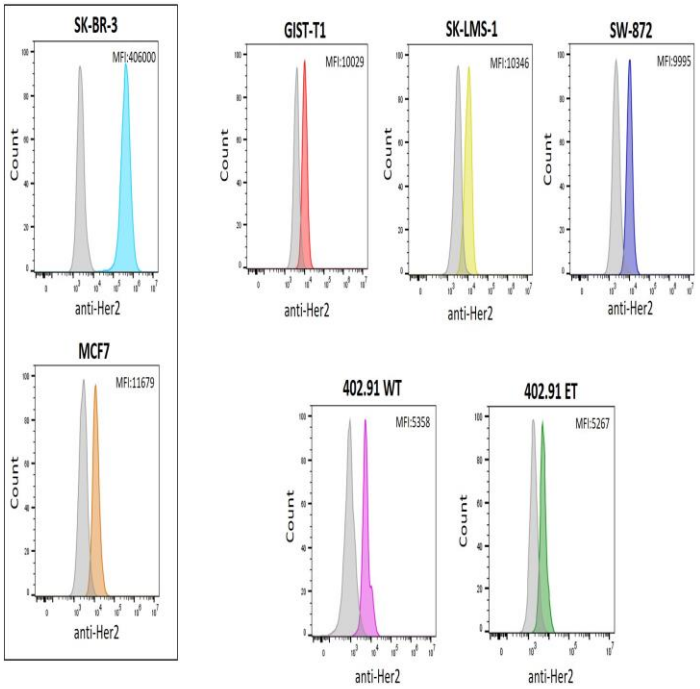
Objective: Limited options with clinical efficacy for advanced Soft Tissue Sarcoma (STS) existed and outcomes in the population with metastatic disease are generally poor. Many studies reported an over-expression of Her2 in osteosarcoma patients associated with an increased risk of tumor metastasis. No data about the role of Her2 as potential target are reported in STS. Thus, we investigated Her2 expression in different STS cell models and the potential anti-tumor effect of trastuzumab deruxtecan (T-Dx), a novel HER2/neu-targeting antibody-drug conjugate (ADC).

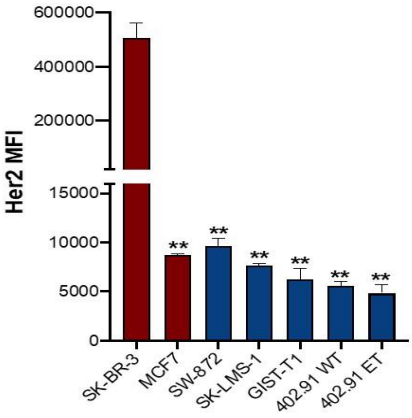
Methods: Her2 expression was evaluated by flow cytometry in different STS cell lines: dedifferentiated liposarcoma (SW-872), leiomyosarcoma (SK-LMS-1), gastrointestinal stromal tumor (GIST-T1), myxoid liposarcoma sensitive (402.91 WT) and resistant to trabectedin (402.91 ET) (Figure 1). As control, we used two breast cancer cell lines with high (SK-BR-3) and low Her2 expression (MCF7) (Figure 1).Tumor cell viability was analyzed after T-Dx treatment by MTT assay.

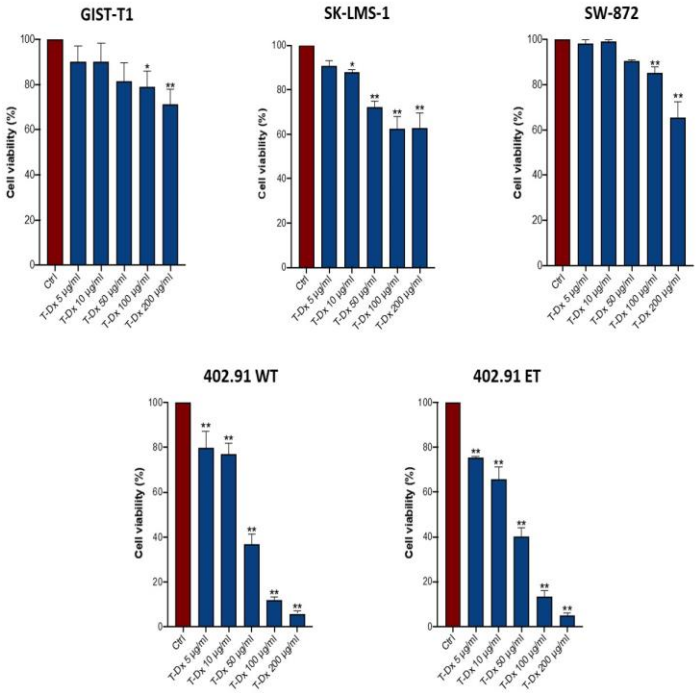
Results: Data showed that all STS cell lines were Her2 positive, but they exhibited low expression levels similar to those observed in MCF7 cells. Her2 median fluorescence intensity (MFI) is reported below: SK-BR-3 (MFI: 505261; SD 56546); MCF7 (MFI: 8735; SD 8978); SW-872 (MFI: 9621; SD 771); SK-LMS-1 (MFI: 7654; SD 245), GIST-T1 (MFI: 6238; SD 1152),

402.91 WT (MFI: 5587; SD 420), 402.91 ET (MFI: 4870; SD 831); (Figure 2). T-Dx treatment significantly affected the viability of all STS, but the rate of cell growth changed in the different cell lines (Figure 3). In particular, 402.91 WT cells were more sensitive to T-Dx treatment, while the lowest anti-tumor effect was found in GIST-T1 cells. Interestingly, a significant T-Dx cytotoxicity was observed also in trabectedin resistant cells (402.91 ET), suggesting its potential efficacy in chemo-resistant tumors (Figure 3).

Conclusion: These results showed, for the first time, an anti-tumor effect of T-Dx in STS providing the rationale for testing this novel HER2-targeting ADC in in vivo STS models.







(P 264) CYCLIN-DEPENDENT KINASE 4/6 INHIBITORS AND TRABECTEDIN IN SOFT TISSUE SARCOMA: A NOVEL POTENTIAL SYNERGY?

Bruno Vincenzi, MD, PhD[1]; Francesco Pantano, MD, PhD[1]; Michele Iuliani, PhD[2]; Sonia Simonetti, PhD[2]; Silvia Cavaliere, PhD student[2]; Alessandro Mazzocca, MD[1]; Giuseppe Tonini, MD, PhD[1], (1) Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Lazio, Italy, (2) Università Campus Bio-Medico di Roma, Rome, Lazio, Italy

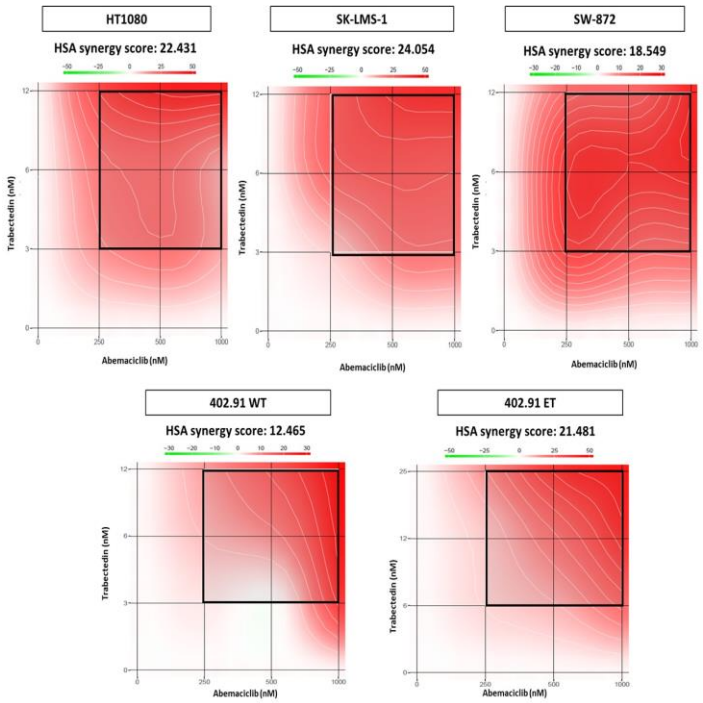
Objective: Trabectedin has a unique mechanism of action that differs from the other available DNA-damaging agents used in cancer chemotherapy. Indeed, trabectedin has a higher cytotoxicity on cells in the G1 phase than other chemotherapy acting on proliferative cells. CDK4/6 inhibitors regulate the G1 restriction cell-cycle checkpoint leading to a cell arrest in G0/G1-phase. Based on these evidences, we evaluated the potential synergistic effect of CDK4/6 inhibitors and trabectedin in different soft tissue sarcoma (STS) cell models.

Methods: The following STS cell lines were used: leiomyosarcoma (SK-LMS-1), dedifferentiated liposarcoma (SW-872), fibrosarcoma (HT1080), myxoid liposarcoma sensitive (402.91 WT) and resistant to trabectedin (402.91 ET). These cells were treated with abemaciclib and trabectedin, alone or in combination, and cell viability was assessed after 4 days by MTT assay. The synergistic effect of drug combination was analyzed by SynergyFinder web application (version 3.0) (Synergy score > 10: the interaction between two drugs is likely to be synergistic).

Results: Data showed a synergistic effect of trabectedin and abemaciclib in SK-LMS-1 (synergy score: 24.054), SW-872 (synergy score: 18.549), HT1080 (synergy score: 22.431) and 402.91 WT cells (synergy score 12.465) (Figure 1). Interestingly, this synergy was found also in

402.91 cells resistant to trabectedin (synergy score 21.481) (Figure 1). Moreover, we tested the combination between abemaciclib and doxorubicin, a chemotherapy with a different mechanism of action. No synergistic effect was observed in STS cell lines, with the exception of a weak synergy found in HT1080 cells (sinergy score: 12.716).

Conclusion: This study demonstrated, for the first time, a synergy between CDK4/6 inhibitors and trabectedin in different STS cells and in trabectedin resistant cells providing the rationale for testing this combination treatment in STS in vivo models.



(P 265) SELECTIVE TARGETING OF BET FAMILY EPIGENETIC REGULATORS IN ADVANCED AND METASTATIC ALVEOLAR RHABDOMYOSARCOMA

Anna Mandel, MSc[1]; Rebecca Gladdy, MD, PhD[2]; Yael Babichev, PhD[3]; Jen Dorsey*, PhD[4]; Claire Wunker, MD, MSc[5]; Abha Gupta, MD MSc[6]; Richard Marcellus, PhD[7]; Brendan C. Dickson, MD[5]; Elizabeth Demicco, MD, PhD[5], (1) University of Toronto, Toronto, ON, Canada, (2) Mount Sinai Hospital, Sinai Health System, and Princess Margaret Cancer Centre Toronto, ON, Canada Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada University of Toronto, Toronto, ON, Toronto, ON, Canada, (3) Sinai Healthcare System, Toronto, ON, Canada, (4) Lunenfeld Tanenbaum Research Institute/Mount Sinai Hospital, Toronto, ON, Canada, (5) Sinai Health System, Toronto, ON, Canada, (6) Princess Margaret Cancer Center, Toronto, ON, Canada, (7) OICR, Toronto, ON, Canada

Objective: Standard of care drugs for rhabdomyosarcoma (RMS), such as vincristine and doxorubicin, have a high systemic toxicity and >70% of high-risk patients develop resistance. Epigenetic modulation has shown promise in predicting fusion-positive (FP) alveolar RMS (ARMS) outcomes. Therefore, our studies focus on this subtype and BET inhibition (BETi) as a

potential therapy. Additionally, we are working on metastatic ARMS mouse models, which better represent high-grade disease compared to primary xenografts. Our goal is to determine what effect BETi has on localized and metastatic ARMS, and whether BETi could be a viable treatment option for these patients.

Methods: ARMS cell lines (RH4, RH30) were treated with BETi compounds (BMS-986158, PLX51107) using a 12-point serial dilution (2.6 nM-10 □M). Half maximal inhibitory concentration (IC50) values were calculated using a sigmoidal curve fit with three parameters following ATPlite viability assay. Caspase-3 activation assay was used to evaluate apoptosis via flow cytometry. The downstream impact of BETi on MYC expression was analyzed using qPCR, and on-target effect was confirmed through IncuCyte live cell imaging after siRNA knockdowns of BETi target proteins BRD2/BRD3/BRD4 were created in the ARMS cell lines. To establish metastatic models, NSG mice were injected with ARMS (RH4, RH30) cells and after tumor growth, amputation was performed on the tumor-bearing hindlimb. Mice were monitored for systemic illness and palpable metastatic or recurrent tumor growth until reaching endpoint, followed by euthanasia and necropsy to assess for metastases. Metastases were stored as frozen sections for transcriptome analysis and in formalin for immunohistochemistry staining. Histological characterization of metastatic tumors was performed by sarcoma pathologist Dr. Brendan Dickson, and PAX3-FOXO1 (P3F) fusion was assessed using RT-PCR. Metastatic tumor-derived cells were tested for BETi sensitivity using a 12-point serial dilution (2.6 nM-10 □M) followed by ATPlite assay.

Results: BETi reduced ARMS proliferation and induced apoptosis in vitro. The oncogenic driver MYC is a known downstream transcriptional target of BET proteins. MYC mRNA levels significantly decreased in ARMS cells treated with BETi, confirming dose efficacy. Depletion of BRD4 hindered proliferation, ensuring target specificity of the compounds (Fig. 1). Metastatic models exhibited metastasis in sites relevant to human disease (liver, lymph nodes, lungs, retroperitoneum) (Fig. 2). Metastatic and primary tumors shared RMS histopathological features. To ensure that the ARMS metastatic tumors reflected the molecular characteristics of the original P3F FP cells, we evaluated the P3F fusion status of the tumors and found that the fusion was preserved, confirming the origin of the metastases (Fig. 3). BETi sensitivity of metastasis-derived cell lines (n=3) was comparable to the original cell lines. This suggests that metastatic RMS might respond well to BETi treatment, indicating the need for further in vivo studies using BETi in our metastatic models.

Conclusion: Functional validation confirmed BETi's potential for in vivo ARMS studies. Ongoing clinical trials of the primary drug candidate, BMS-986158, in childhood cancers increases the likelihood of clinical trial opportunities in RMS. Our metastatic models demonstrate clinical relevance and BETi sensitivity, crucial for addressing the lack of established metastatic ARMS studies. Next, we will compare transcriptomes of metastatic tumors and primary xenografts. With the help of our collaborators, we aim to cluster metastatic tumors with large-scale patient data, potentially informing patient study stratification. Ultimately, our in vivo studies will assess BETi's impact on preventing metastasis and reducing metastatic burden.

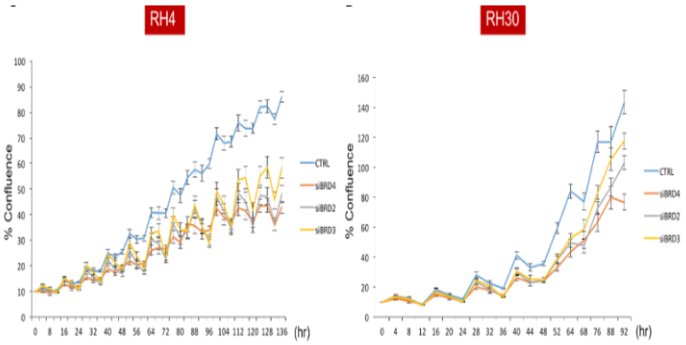


Figure 1: Testing the on-target specificity of the BETi compounds in ARMS. RH4 and RH30 cells were transfected with 50 nM mock, BRD4, BRD2 or BRD3 siRNA for 48 hours in technical triplicates and were allowed to grow until the vehicle-treated cells reached confluency. Cell were plated to achieve 10% confluence at the time of siRNA transfection. Confluence was quantified from phase contrast images taken every 4 hours using the IncuCyte ZOOM in 96-well plate format. Those time-points where the instrument was out-of-focus due to the low contrast appearance of the cells were excluded. n=3 biological repeats were performed with both cell lines.

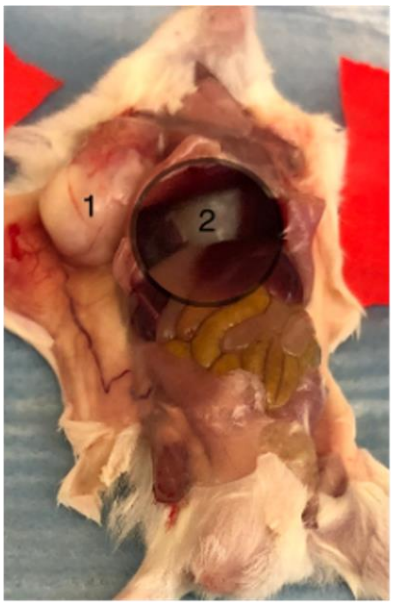


Figure 2: Necropsy images of axillary (1) and liver (2) metastases in RH4 xenograft mice.

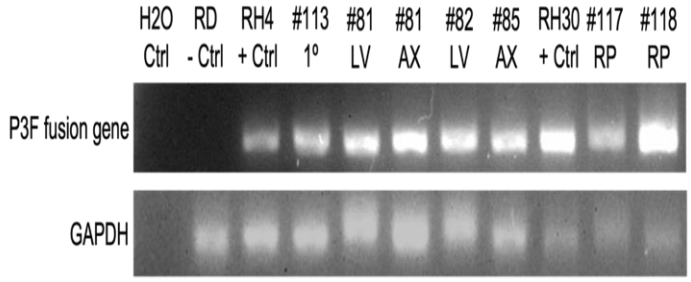


Figure 3. PAX3-FOXO1 fusion status of ARMS primary xenograft and metastatic models. RT-PCR was conducted to assess the PAX3-FOXO1 fusion status of tumors originating either from the primary xenograft site or various metastatic sites of RH4 and RH30 mouse models. Water served as baseline control, and RD, a fusion negative ERMS cell line, as negative control. RH4 and RH30 commercial cell lines served as positive control. RH4 mouse models are denoted with #113, #81, #82 and #85. RH30 mouse models are denoted with #117 and #118. Abbreviations: AX=axillary metastasis, Ctrl=control, LV=liver metastasis, P3F=PAX3-FOXO1, RP=retroperitoneal metastasis

(P 266) ROMIDEPSIN DECREASES DNA DAMAGE REPAIR AND INCREASES SENSITIVITY TO CHEMOTHERAPY IN EWING SARCOMA

Erin Trovillion, MD[1]; Kaitlyn Smith, PhD[2]; Divya Gandra, BS[2]; Karl Dykema, n/a[2]; Abhinav Nagulapally, n/a[2]; Chloe Sholler, n/a[2]; Javier Oesterheld, MD[2], (1) Atrium Health - Levine Children's Hospital, Charlotte, NC, (2) Atrium Health Levine Children's Hospital, Charlotte, NC

Objective: Treatment options for Ewing sarcoma (EWS) patients have not improved in several decades; the poor survival rates, especially for those with metastatic and relapsed disease, highlight the need for improved therapeutic options. Through sequencing of patient tumors, we have identified HDAC2 as a potential therapeutic target for treating EWS. Our aims are to evaluate the efficacy and mechanism of Romidepsin monotherapy and in combination with standard of care chemotherapeutic agents in Ewing sarcoma cells in vitro and in vivo Ewing sarcoma xenograft mouse models.

Methods: EWS cells were treated with a range of clinically relevant concentrations of Romidepsin alone or in combination with standard of care chemotherapy agents and cell viability was measured by CellTiter-Glo. SynergyFinder 3.0 was used to determine if there is a synergistic response. To investigate the mechanism of Romidepsin, cells were treated with drug and western blotting was used to look at changes in proteins related to cell cycle, DNA damage response, and apoptosis.

Results: Romidepsin was cytotoxic to all Ewing sarcoma cell lines tested with clinically relevant IC50s (< 10nM). Romidepsin synergizes with Doxorubicin and etoposide, and significantly decreases cell viability when added to standard chemotherapy combinations at clinically relevant doses. Romidepsin represses expression of Cyclin D1, CHEK1, and CHEK2, and

increases cleavage of Caspase 3.

Conclusion: HDAC inhibition shows a decrease in cell viability in EWS cell line models alone and when combined with doxorubicin or etoposide. The addition of Romidepsin to chemotherapy combinations significantly decreases cell viability compared to that of chemotherapy alone, suggesting that the addition of Romidepsin to patient care may lead to improved outcomes. Mouse models are in development to further test in vivo and mice will be monitored for tumor growth and survival.

(P 267) NEW MOUSE MODEL OF SYNOVIAL SARCOMA BASED ON CONDITIONAL EXPRESSION IN MESENCHYMAL PROGENITORS

Xue Q. Wang, BSc[1]; Wilder Scott, PhD[2]; Lauren A. Martin, MSc[2]; Martin Arostegui, PhD[2]; Martin Hirst, PhD[3]; Kevin B. Jones, MD[4]; Michael Underhill, PhD[2]; Torsten O. Nielsen, MD, PhD[5], (1) University of British Columbia, Vancouver, BC, Canada, (2) Biomedical Research Centre, University of British Columbia, Vancouver, BC, Canada, (3) Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada, (4) University of Utah, Salt Lake City, UT, (5) Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Objective: Conditional mouse models are an important strategy to uncover cancer biology and develop new treatments. However, despite recent advancements in our understanding of synovial sarcoma biology and its SS18SSX fusion oncogene driver, the cell of origin (in which conditional expression of SS18SSX would best model the disease) remains undefined. We hypothesize that Hic1+ mesenchymal progenitor cells may be relevant cells of origin for synovial sarcoma.

Methods: We previously generated a specific Hic1CreERT2 system that has enabled us to identify mesenchymal progenitors, characterize and define their lineage trajectories through embryonic development into adulthood. This mesenchymal progenitor-specific Cre line has been used to generate a unique mouse model of synovial sarcoma where the human SS18-SSX fusion oncoprotein (with an EGFP marker) is conditionally expressed in Hic1 (Hypermethylated in cancer 1)-expressing mesenchymal progenitor cells. To generate this model, a genetically engineered mouse model was created by crossing the Hic1CreERT2 driver with a floxed SS18-SSX2 allele. Oncoprotein expression is induced in 8-week old mice by administration of tamoxifen (which enables CreERT2 to enter the nucleus).

Results: This model shows robust formation of sarcomas with 100% penetrance after a mean latency of 15.5 weeks. Histologic analyses of tumors reveal characteristic cellular morphology reflective of the three major sub-types of synovial sarcoma, expressing key markers including human SS18SSX and mouse TLE1 and BCL2 proteins. Tumors primarily form in two sites: the stifle joints and the tongue. The orthotopic sarcomas in the stifle are most commonly monophasic and can be found in ~ 90% of mice at endpoint. Tumors in the tongue are faster growing, present with the histology of poorly differentiated biphasic synovial sarcomas, and are found in 100% of mice. These tongue tumors allow for robust, accessible, and replicable monitoring of tumor development. Microscopic precursor lesions are detectable in the tongue by 4 weeks post-tamoxifen induction, are grossly visible at 8 weeks allowing for ready monitoring of tumor growth and drug response, and result in endpoint on average at 16 weeks post-induction. Single-cell RNA-seq gene expression profiles of these tumors recapitulate

orthologous genes expressed in human synovial sarcoma, highlighting the genetic programs involved in sarcomagenesis.

Conclusion: This new mouse model of synovial sarcoma closely recapitulates human disease as assessed by tumor location, gene expression, diagnostic markers, and histomorphology, identifying a subset of Hic1+ mesenchymal progenitor cells as permissive cells of origin for this sarcoma. The robustness and predictability of the tumor formation in this model opens a unique opportunity to test novel compounds for their therapeutic potential in synovial sarcoma.

(P 268) IMPACT OF CELLULAR FUSION IN THE ONCOGENESIS OF LEIOMYOSARCOMAS

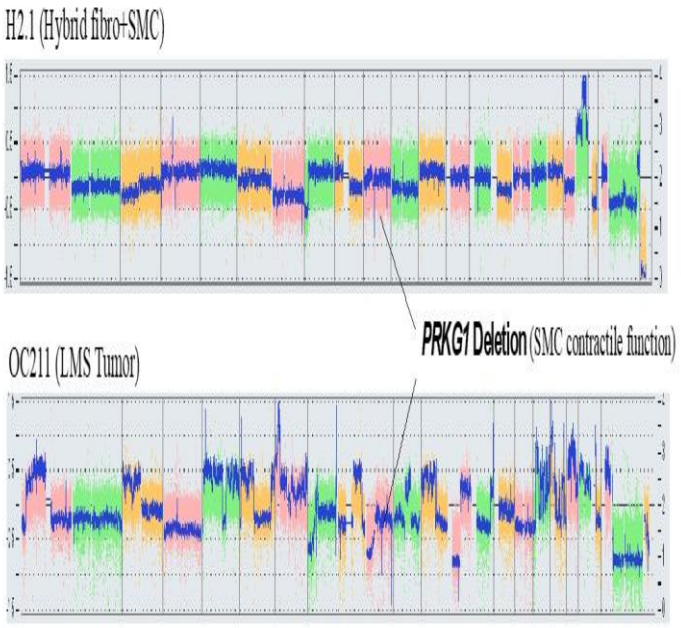
Ariadna Brito, Master[1]; Gaëlle Perot, PhD[1]; Natacha Roussel, Master[1]; Lucile Delespaul, PhD[1]; Philippe Rochaix, MDPhD[2]; Gwenael Ferron, MD, PhD[3]; Frederic Chibon, PhD[1], (1) Cancer Research Center of Toulouse, Toulouse, Midi-Pyrenees, France, (2) Oncopole Claudius Regaud, Toulouse, Midi-Pyrenees, France, (3) Oncopole, Toulouse, Midi-Pyrenees, France

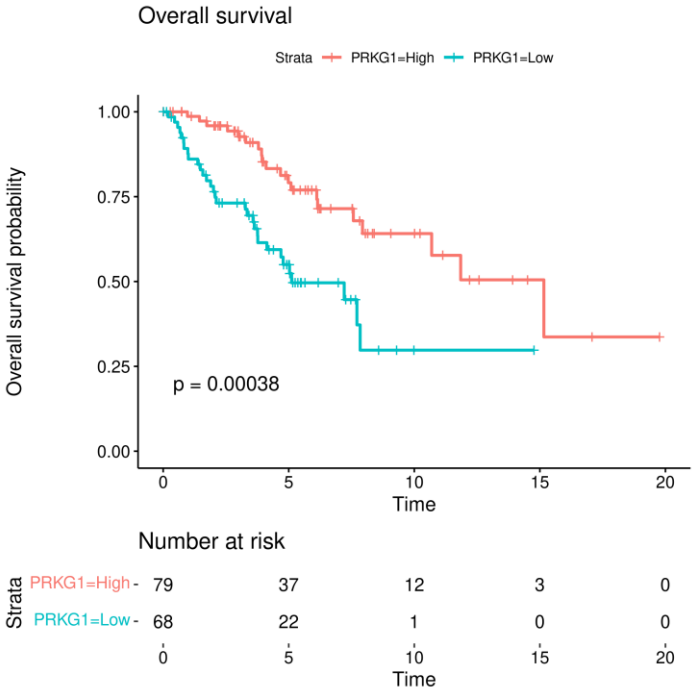
Objective: Leiomyosarcoma (LMS) is one of the most common and aggressive type of soft tissue sarcomas. Around 50% of the patients develop metastasis translating in an overall survival rate of 12 months after the dissemination of the disease. LMS are very heterogeneous and they show highly rearranged genome with no recurrent alterations. The treatment options for LMS are still restricted, the development of deeper knowledge in LMS genomics could uncover key biological features for the design of new therapeutically strategies. While whole genome doubling is very frequent in LMS oncogenesis, the mechanism leading to tetraploidization is unknown. We hypothesized that cell fusion is one of the mechanisms contributing to LMS oncogenesis, heterogeneity and chaotic genome.

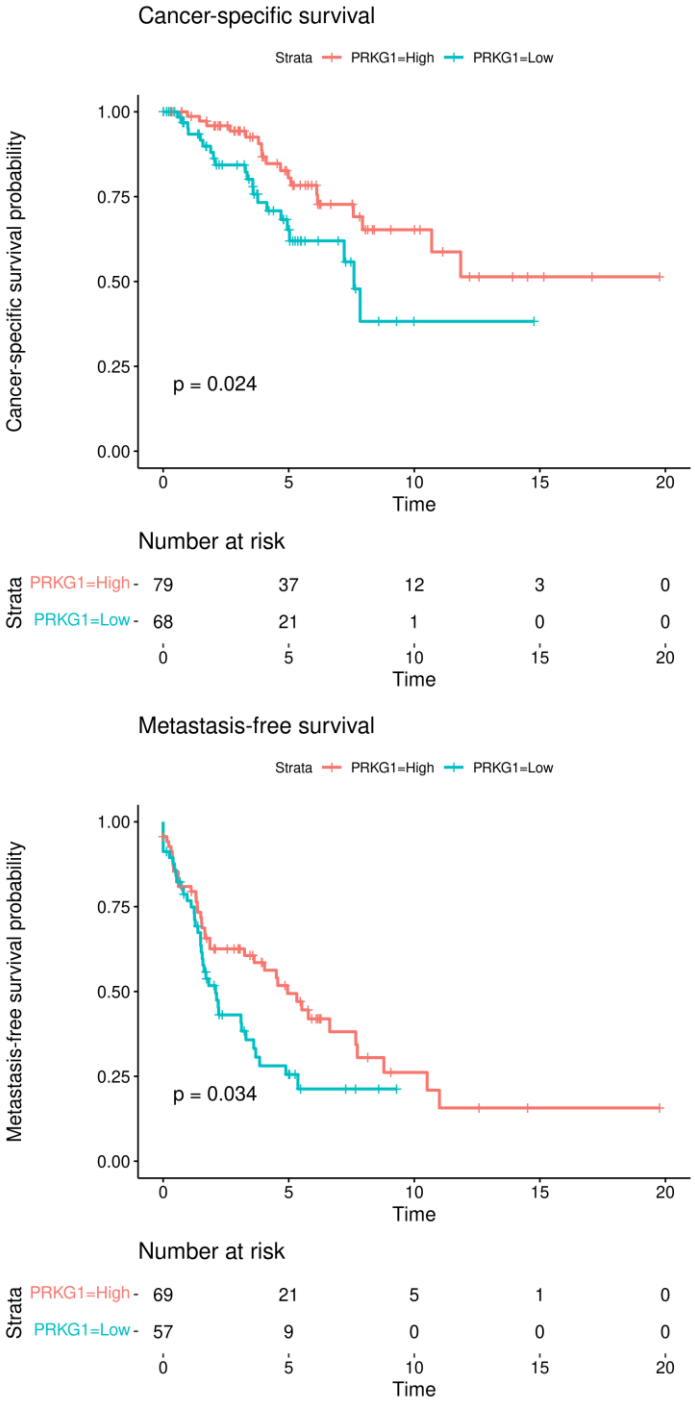
Methods: To asses this hypothesis, we produced a spontaneous cell fusion model between fibroblast and smooth muscle cells, in which we observed, additional to a highly rearranged genome, a very specific break point deletion in chromosome 10, in the gene PRKG1. PRKG1 is involved in smooth muscle contractile function and cellular proliferation. We also use primary LMS cell lines with PRKG1 deletion to unveil the different cell lines' phenotypes. To further study the frequency and role of PRKG1, we analyze whole genome sequencing (WGS), comparative genome hybridization (CGH) and RNA sequencing from LMS tumor samples and cell lines.

Results: After studying a cohort of 123 LMS patients in whole genome sequencing (WGS), we observed that the deletion of PRKG1 was frequent (14%). This makes PRKG1 be among the five most commonly altered genes in LMS. Phenotypic analysis were performed comparing cell lines where PRKG1 was deleted with their overexpress counterparts. Results show that when PRKG1 is deleted cell lines have a higher migration rate. Low PRKG1 expression is also associated with poor overall and metastatic survival of LMS patients.

Conclusion: In conclusion, with this cell fusion model we revealed that cell fusion could be one of the mechanisms involved in the development of LMS through genome reshuffling. PRKG1 deletion/down regulation, triggered by genome merging, is therefore associated with tumor evolution.


H2.1 (Hybrid fibro+SMC)
PRKG1 Deletion (SMC contractile function)
OC211 (LMS Tumor)


Overall survival
Strata PRKG1=High PRKG1=Low
Overall survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.00038
0
5
10
15
20
Time
Number at risk
Strata PRKG1=High 79 37 12 3 0
PRKG1=Low 68 22 1 0 0
0
5
10
15
20
Time


Cancer-specific survival
Strata PRKG1=High PRKG1=Low
Cancer-specific survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.024
0
5
10
15
20
Time
Number at risk
Strata PRKG1=High 79 37 12 3 0
PRKG1=Low 68 21 1 0 0
0
5
10
15
20
Time
Metastasis-free survival
Strata PRKG1=High PRKG1=Low
Metastasis-free survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.034
0
5
10
15
20
Time
Number at risk
Strata PRKG1=High 69 21 5 1 0
PRKG1=Low 57 9 0 0 0
0
5
10
15
20
Time

(P 269) PLASMA DNA METHYLATION-BASED BIOMARKERS FOR MPNST DETECTION IN PATIENTS WITH NEUROFIBROMATOSIS TYPE 1 (NF1)

Keila E. Torres, MD, PhD, MBA, FACS[1]; Katarzyna Tomczak, Ph.D[1]; Manishkumar S. Patel, PhD[2]; Christine B. Peterson, PhD[1]; Sharon Landers, PhD[1]; Angela D. Bhalla, PhD, MPH[1]; Jace P. Landry, MD[1]; Carson Callahan, MD, PhD[1]; Alexander J. Lazar, MD, PhD[1]; Ian McCutcheon, MD[1]; John M. Slopis, MD,MPH[1]; Andy Livingston, MD, MS[1]; B Ashleigh Guadagnolo, MD,MPH[1]; Di Zhang, MS[1]; Justin Wong, Ph.D.[1]; Emily Z. Keung, MD, AM, FACS[1]; Christopher Scally, MD[1]; Christina Roland, MD, MS[1]; Kelly Hunt, MD[1]; Jian Gu, Ph.D[1]; Paul Scheet, Ph.D[1]; Liang Wang, Ph.D[3]; Kunal Rai, PhD[1], (1) The University of Texas MD Anderson Cancer Center, Houston, TX, (2) Moffitt Cancer Center & Research Institute, Tampa, FL, (3) Moffitt Cancer Center & Research Institute, Tampa Bay, FL

Objective: A significant challenge in caring for individuals with NF1 is the ability to diagnose MPNSTs early when the tumors can be surgically removed. Currently, clinicians use periodic cross-sectional imaging modalities such as computerized tomography (CT) and magnetic resonance imaging (MRI) to monitor patients. Although these existing imaging modalities can define the anatomic extent of lesions, they cannot reliably distinguish MPNSTs from benign plexiform neurofibromas (PNs). Further, 18F-FDG PET is associated with radiation exposure, and false positive results in plexiform neurofibromas have been described to occur at a rate of 5%. Consequently, these patients need confirmation of malignant transformation from a histological analysis requiring a biopsy. In light of the critical need for early diagnosis of MPNST, developing accurate and less invasive screening modalities for patients with NF1 and MPNSTs is imperative. To address this challenge, we identified and validated a panel of plasma-derived circulating cell-free DNA (cfDNA) methylation biomarkers.

Methods: Genome-wide DNA methylation profiling (Infinium HumanMethylation450 BeadChip assay, Illumina) of a discovery cohort of 13 patients with MPNSTs (29 samples of tumor and adjacent neurofibroma tissues) and of NF1-associated MPNST cell linesand controls (n=3) was performed to identify and validate candidate MPNST-specific CpG sites (CpGs). We constructed a logistic regression prediction model to select MPNST-specific CpGs distinct from adjacent neurofibromas and normal tissue samples. We utilized bisulfite sequencing and/or ddPCR methods to confirm the presence of hypermethylation of candidate CpGs across validation cohort (MPNST n=10, NFs n=12 tissues) and cell lines (MPNST n=2, Normal Schwann cells n=2). After validation of the candidates in tissue and cell lines we then evaluated if hypermethylation at selected CpG sites can also be detectable in plasma samples from patients with MPNST. For this we applied cfMBD-seq to profile the cfDNA methylome of blood samples from patients with MPNST (n=6) and patients with NF1 (n=6).

Results: Our comprehensive DNA methylation analysis and validation of selected differentially methylated regions confirmed the presence of a characteristic hypermethylation pattern during MPNST development in human samples and in an in vitro model. Importantly, based on stringent feature-selection criteria and the application of predictive modeling, we generated a list of 73 candidate MPNST-specific CpGs showing good performance in discriminating MPNSTs from neurofibromas. Validation of 5 candidate biomarkers confirmed successful MPNST detection (sensitivity: > 88%, specificity: > 91%) in analyzed tissue. Analysis of plasma samples demonstrated that 70 of 73 selected genomic regions with greater than 1.2-fold higher methylation in MPNST patients than NF1 patients. Further, we identified 16 CpG islands that consistently separated plasma from patients with confirmed MPNST diagnosis from samples of individuals with NF1 who did not have a diagnosis of malignant transformation (FDR < 0.1).

Conclusion: Our findings confirmed the presence of a unique hypermethylation pattern during malignant transformation. Further, 16 of the hypermethylated CpG sites from tumor-based selection criteria were validated in plasma samples, highlighting the great potential to be used as biomarkers in clinical settings for early MPNST detection in NF1 patients.

(P 270) ALTERNATIVE SPLICING (AS) REGULATION AS A NOVEL THERAPEUTIC TARGET IN SOFT-TISSUE SARCOMA (STS)

David S. Moura, PhD[1]; Esther Martinez-Font, PhD[2]; Carine Bossard, PhD[3]; Antonio Rubio-Reyes, Lab technician [4]; Jose L. Mondaza-Hernandez, Master degree[1]; Marta Martín-Ruiz, PhD[5]; Pablo Romero, Master degree[1]; Marta Renshaw, Technician lab[1]; Jaime Carrillo-Garcia, PhD[6]; Davide Di Lernia, MD[1]; Nadia Hindi, MD, MSc[7]; Antonia Obrador-Hevia, PhD[2]; Javier Martin-Broto, MD, PhD[7], (1) Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM),, Madrid, Madrid, Spain, (2) Fundació Institutd'InvestigacióSanitàriaIlles Balears –IdISBa, Mallorca, Islas Baleares, Spain, (3) BioSplice, San Diego, CA, (4) Research health institute of Fundacion Jimenez Diaz, Madrid, Madrid, Spain, (5) Instituto de Investigaciones Sanitarias Fundación Jiménez Díaz, Madrid, Madrid, Spain, (6) Research Health Institute of Fundación Jiménez Díaz, Moralzarzal, Madrid, Spain, (7) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain

Objective: There is an urgent clinical need to identify novel treatments for advanced STS, since all the clinical trials testing chemotherapy combinations have failed to improve survival compared to doxorubicin (dox) alone. AS deregulation has been associated with cancer initiation, progression and resistance to therapy. This deregulation can occur due to mutations or imbalanced expression or activity of splicing factors (SF). The alteration SF can alter thousands of pre-mRNAs, increasing cellular complexity and facilitating tumour progression. CDC-like kinases (CLK) that phosphorylate serine-arginine-rich SF (SRSFs), are key regulators of AS. SM09419 is an oral pan CLK/DYRK inhibitor that has been shown to inhibit SRSF phosphorylation, selectively modify spliceosome activity and decrease tumour growth by multiple mechanisms including the downregulation of Wnt/β-catenin signalling activity, a pathway that we reported to be important in sarcoma.

Methods: A panel of 9 STS cell lines, including leiomyosarcoma (CP0024, IEC005, AA and SK-UT-1), liposarcoma (93T449), synovial sarcoma (MCP037) and UPS (MCP016, MCP021 and MCP025) were used to analyse the effect of SM09419 (provided by Biosplice) or PRI-724 (Wnt/β-catenin inhibitor) treatment on cell viability (MTS assays). TOP flash β-catenin/TCF-responsive reporter assay was performed to determine the transcriptional inhibition of the Wnt/β-catenin pathway in response to SM09419. In vivo experiments tested the combination of SM09419 (25mg/kg 5 days on and 2 off, for 21 days cycle) plus IP dox (5mg/kg once a week for 21 days cycle) in leiomyosarcoma (IEC005) and synovial sarcoma (FJD-SS-001) PDX models.

Results: Our results showed that SM09419 had superior activity in STS cells compared to specific Wnt/β-catenin inhibitors with IC50 values starting at 29.8 nM in SK-UT-1 cell line, whereas PRI-724 IC50 values were clearly higher, starting from 5.18 µM in 93T449 cell line.

SM09419 treatment significantly inhibited the transcriptional activity of β-catenin by 40% in the MCP021 cell line, demonstrating the regulatory activity of AS inhibitors on this pathway. In vivo studies confirmed the higher efficacy of SM09419 in monotherapy compared to dox, in inhibiting tumour growth ($p < 0.05$), which was potentiated when combined with dox. The combination was increasingly active in synovial sarcoma with tumour regressions in 3 out of 4 mice, including one complete response. Treatment schedule was adapted to a single IP administration of dox (5mg/kg) and 5 administrations of SM09419 (25mg/kg), to improve the tolerability of the combination.

Conclusion: The effects of pan CLK/DYRK inhibition on growth and survival of STS cancer cell lines and PDX models, indicate that can STS can potentially be therapeutically addressed with these AS-modulators. The combination of SM09419 with dox was active in STS preclinical models known for their resilience toward treatment. Based on these results, the exploration of such combinations in clinical trials is warranted.

(P 271) PHARMACODYNAMICS AND MECHANISTIC IMPACTS OF FHD-609, A BROMODOMAIN-CONTAINING PROTEIN 9 DEGRADER, IN A PHASE 1 STUDY IN PATIENTS WITH ADVANCED SYNOVIAL SARCOMA OR SMARCB1-LOSS TUMORS

Mike Collins, PhD[1]; Ariel Lefkovith, MA[1]; Jessica Wan, BS[1]; Kim Horrigan, BS[1]; Salih Topal, PhD[1]; David L. Lahr, PhD[1]; Alexandra R. Ballesteros-Perez, PhD[2]; Alexander J. Lazar, MD, PhD[3]; Kathleen Healy, n/a[1]; Sarah Reilly, MD, MPH[4]; Samuel V. Agresta, MD[5]; Scott Innis, MS[1]; Jessica Piel, PhD[1], (1) Foghorn Therapeutics, Cambridge, MA, (2) Certara, Richmond Hill, GA, (3) The University of Texas MD Anderson Cancer Center, Houston, TX, (4) Foghorn Therapeutics, Boston, MA, (5) Foghorn Therapeutics, Lexington, MA

Objective: Bromodomain-containing protein 9 (BRD9) represents a potential selective vulnerability for tumors with specific alterations in the Brahma-associated factor (BAF) chromatin remodeling complex. Incorporation of the SS18-SSX oncoprotein into BAF complexes, or recurrent loss-of-function mutations in the core BAF subunit SMARCB1 drive tumorigenesis and may lead to dependence on non-canonical BAF (ncBAF) for survival. Because BRD9 is a complex-specific subunit of ncBAF, selective BRD9 degradation provides a potential therapeutic approach to treat these diseases. FHD-609 is a potent, selective, heterobifunctional degrader of BRD9 investigated in BRD9-dependent cancers, including synovial sarcoma and solid tumors primarily characterized by or with evidence of SMARCB1 loss. The pharmacodynamics of FHD-609 were assessed in a Phase 1 study in order to demonstrate proof-of-mechanism and to aid in the identification of the recommended Phase 2 dose(s). The downstream molecular and phenotypic impacts of prolonged BRD9 degradation in tumor tissue were also explored.

Methods: BRD9 protein levels were detected in patient tumor biopsies and peripheral blood mononuclear cells (PBMCs) using our previously reported immunohistochemistry (IHC) and flow cytometry methods, respectively. Biopsies were collected at screening and either Cycle 2 Day 15 (C2D15), C3D1 or end of treatment, while PBMCs were isolated from serial blood samples. Additional relevant biomarkers, including Ki67, c-Myc and SS18-SSX were also evaluated by IHC. Phenotypic impacts of FHD-609 treatment were assessed by histology, and transcriptional changes were explored by RNA sequencing.

Results: BRD9 levels were assessed in tumor biopsies from 8 different dose cohorts, ranging from 5 mg to 80 mg twice-weekly (BIW), as well as 80 mg and 120 mg once-weekly (QW). Dose-dependent BRD9 degradation was observed in tumor cells, with H-scores of 0 in several samples at doses of 40 mg BIW and above. In PBMCs, all dose levels of FHD-609 achieved approximately the same degree of BRD9 degradation at the earliest timepoint (30 minutes post-infusion). However, the duration of degradation was extended at higher dose levels. The downstream impacts of BRD9 degradation in tumor tissue were also investigated. In several biopsies with BRD9 H-scores of 0, the percentage of tumor cells positive for the proliferation marker Ki67 was markedly reduced relative to screening. Histological changes were also observed, with more pronounced treatment effects generally occurring at higher dose levels. Notable findings included reduced cellularity, altered cellular morphology including small cell change, and altered tissue architecture in some cases. Finally, RNA sequencing of tumor biopsies revealed broad transcriptional impacts in response to FHD-609 treatment. Notably, gene set enrichment analysis identified dose-dependent downregulation of gene sets associated with oncogenic growth and proliferation.

Conclusion: In this Phase 1 dose escalation study, treatment with FHD-609 drove robust degradation of BRD9 in synovial sarcoma patient tumors. Complete degradation was observed in samples from QW and BIW cohorts, including several collected multiple days post-dose. BRD9 degradation was associated with histological treatment effects, and decreased markers of proliferation.

(P 272) A COMPARATIVE ASSESSMENT OF SELINEXOR WITH TRABECTEDIN OR LURBINECTEDIN IN PATIENT-DERIVED XENOGRAFTS (PDX) OF DEDIFFERENTIATED LIPOSARCOMA (DDLPS)

Sandro Pasquali, MD, PhD[1]; Noemi Arrighetti, PhD[2]; Valentina Zuco, N/A, MSc[2]; Cesare Soffientini, MSc[2]; Monica Tortoreto, N/A, MSc[2]; Silvia Brich, N/A, PhD[2]; Marta Barisella, MD[3]; Paola Collini, MD[4]; Chiara Fabbroni, MD[5]; Sara Cingarlini, MD[6]; Alessandro Gronchi, MD, FSSO[7]; Silvia Stacchiotti, MD[8]; Roberta Sanfilippo, MD[9]; Nadia Zaffaroni, PhD[10], (1) Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (2) Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Lombardia, Italy, (3) Department of Pathology,Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (4) Department of Advanced Diagnostics , Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (5) Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (6) University of Verona, Verona, Veneto, Italy, (7) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (8) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (9) Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Lombardia, Italy, (10) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy

Objective: Clinical trials showed the limited efficacy of trabectedin or selinexor as single agent in patients with metastatic DDLPS. We conducted a preclinical study on two patient-derived xenografts (PDXs) of DDLPS to test the activity of combined and sequential treatment with selinexor and trabectedin or lurbinectedin, following anecdotal evidence of clinical benefit from a rechallenge to trabectedin after selinexor in two patients with metastatic DDLPS. Two patients affected by metastatic DDLPS were re-challenged to trabectedin after developing disease

progression during treatment with selinexor and they did show a partial response to trabectedin.

Methods: Two PDXs (LPS-BZ; LPS-NV) from two primary localized DDLPS treated with surgery at Fondazione IRCCS Istituto Nazionale dei Tumori (Milan, Italy) were established in SCID mice. These models represented the DD component of DDLPS and were histomorphologically and molecularly (by FISH and RNAseq) characterized for consistency with the originating clinical tumors. Firstly, we tested the anti-cancer activity of either selinexor or trabectin or their sequence (selinexor followed by trabectedin). Secondly, we considered luribinectedin, a trabectedin analogue, which we analyzed as single agent or together with selinexor, both in a sequential (selinexor-lurbinectedin) and concomitant sequence. Drug activity was evaluated as maximum tumor volume inhibition percentage (max TVI%).

Results: The anti-cancer activity of a re-challenge to trabectedin following selinexor observed in two patients let to the design of in-vivo experiments on PDXs of DDLPS to investigate the activity of a sequential treatment with trabectedin/luribinectedin plus selinexor or their combination. The histomorphological and molecular characteristics of PDXs of DDLPS (LPS-BZ and LPS-NV) were confirmed to recapitulate the clinical tumors. In the PDX model (LPS-BZ) with the faster growth rate (doubling time: 5 days), the sequence of selinexor followed by trabectedin was more effective (max TVI 61%) than each single-agent (maxTVI 46% and 33% for selinexor and trabectedin, respectively). A further experiment was conducted in a second PDX model (LPS-NV) characterized by a slower growth rate (doubling time: 12 days) and results consistently showed a greater activity for the selinexor and trabectedin sequence (mTVI 73%) compared to selinexor (mTVI 53%) or trabectedin (mTVI 35%) alone. Finally, we tested lurbinectedin instead of trabectedin in the LPS-NV model. The sequence selinexor and lurbinectedin (mTVI 81%) or their concomitant combination (mTVI 74%) showed a greater anti-tumor effect compared to single agent selinexor (mTVI 65%) and lurbinectedin (mTVI 24%). RNA-seq analysis of control and treated tumors is ongoing to investigate the determinants of the positive interactions between trabectedin/lurbinectedin and selinexor as a function of treatment schedule.

Conclusion: The combination of selinexor with either trabectedin or lurbinectedin in DDLPS showed anti-tumor activity. Further analyses are ongoing to investigate the mechanisms underpinning the interaction of these drugs.

(P 273) COMPARISON OF A PATIENT-DERIVED XENOGRAFTS (PDX) OF MYXOID PLEOMORPHIC LIPOSARCOMA (MPL) AND PLEOMORPHIC LIPOSARCOMA (PL) HIGHLIGHTS THE EFFECTIVENESS OF ERIBULIN IN A PDX OF MPL

Sandro Pasquali, MD, PhD[1]; Valentina Zuco, N/A, MSc[2]; Monica Tortoreto, N/A, MSc[2]; Silvia Brich, N/A, PhD[2]; Marta Barisella, MD[3]; Paola Collini, MD[4]; Ugo Pastorino, MD[5]; Alessandro Gronchi, MD, FSSO[6]; Patrizia Gasparini, N/A, PhD[5]; Andrea Ferrari, MD[2]; Michela Casanova, MD[2]; Silvia Stacchiotti, MD[7]; Nadia Zaffaroni, PhD[8], (1) Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (2) Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Lombardia, Italy, (3) Department of Pathology,Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (4) Department of Advanced Diagnostics , Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (5) Fondazione IRCCS Istituto Nazionanale dei Tumori, Milano, Lombardia, Italy, (6) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (7) Department of Medical Oncology 2, Fondazione IRCCS Istituto

Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (8) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy

Objective: MPL is an ultra-rare sarcoma usually occurring in children and adolescents, which is characterized by mixed histological features of myxoid and pleomorphic liposarcoma, without either DDIT3 rearrangements or MDM2/CDK4 amplification. MPL is characterized by high risk of disease recurrence and lack of disease-specific therapeutic options other than those used for soft tissue sarcomas (STS). We successfully generated a PDX of MPL to comparatively assess the effectiveness of standard therapeutic options for STS as well as other anti-cancer agents that showed activity in dedifferentiated liposarcoma. Interestingly, drugs such as eribulin may have higher effectiveness in patients with pleomorphic liposarcomas compared to those affected by dedifferentiated liposarcomas. In addition, we exploited a PDX of PL to search for differential response to these treatments with respect to MPL.

Methods: A PDX from a primary locally advanced MPL was generated from a patient pretreated with doxorubicin plus ifosfamide (4 cycles) followed by ifosfamide monotherapy (3 cycles) plus radiotherapy (54Gy) and surgery. We also generated a PDX from a patient with primary localized abdominal pleomorphic liposarcoma of the mesenteric-retroperitoneal space who underwent surgery. These models were histomorphologically and molecularly (by RNAseq) characterized for consistency with the originating clinical tumors. Antitumor activity of single-agent doxorubicin, ifosfamide, trabectedin, eribulin and selinexor as well as the combination of doxorubicin plus ifosfamide was tested in each model. Drug activity was evaluated as maximum tumor volume inhibition percentage (max TVI%).

Results: PDXs of MPL and PL reproduce characteristics of the clinical tumors. In the PDX model of MPL, eribulin achieved complete tumor response in all treated PDX (max TVI 100%). Six tumors regrew after approximately 50 days from the end of treatment while a tumor demonstrated complete histopathological response at the end of the experiment. Although single-agent doxorubicin or ifosfamide (max TVI 87% or 94%, respectively) as well at their combination (max TVI 97%) showed meaningful activity, tumor growth restated after the end of treatment in all mice. Single agent trabectedin (max TVI 67%) and selinexor (max TVI 55%) had limited effectiveness. Conversely, the PDX model of PL showed different tumor responses to these same treatments with eribulin (max TVI 41%) showing almost negligible effect and similar findings were obtained with either selinexor (max TVI 42%) or doxorubicin (max TVI 25%). Single-agent trabectedin (max TVI 80%) or ifosfamide (max TVI 83%) effectively induced tumor response and the addition of doxorubicin to ifosfamide let to the highest tumor response (max TVI 89%), which resulted in tumor growth stabilization until the end of the experiment. The differences in tumor response to eribulin seem to rely on the ability of the drug to selectively induce programmed cell death in terms of both apoptosis and necroptosis in the MPL PDX but not in the PL PDX. Further analysis with RNA-seq is in progress to clarify the mechanisms underpinning the different responsiveness profiles.

Conclusion: To the best of our knowledge this is the first PDX model of MPL and this study suggests the potential relevance of eribulin for this disease. In addition, the comparison with a PDX model of PL highlights different response to treatment approaches, such as the first line treatment options for soft tissue sarcomas doxorubicin with or without ifosfamide. Further analyses are ongoing to understand the determinants of eribulin activity in MPL

(P 274) TYK2 AND MEK COMBINATION THERAPY IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS AND OTHER SOFT TISSUE SARCOMAS

Dana C. Borcherding, PhD[1]; Neha Amin, n/a[1]; Kevin He, BS[1]; Xiaochun Zhang, MD[1]; Yang Lyu, PhD[1]; Carina Dehner, MD PhD[2]; Himanshi Bhatia, PhD[1]; Angad Gothra, n/a[1]; Layla A. Daud[3]; Leliftu Ahmed, n/a[1]; Peter Ruminski, MS[4]; Christine A. Pratilas, MD[5]; Kai M. Pollard, n/a[6]; R Taylor Sundby, MD[7]; Brigitte Widemann, MD[7]; Angela Hirbe, MD,PHD[1], (1) Washington University in St. Louis, St. Louis, MO, (2) Indiana University, Indianapolis, IN, (3) Indiana University , Bloomington, IN, (4) Washington University in St. Louis, St Louis, MO, (5) Johns Hopkins University, Baltimore, MD, (6) John Hopkins University, Reisterstown, MD, (7) National Cancer Institute, Bethesda, MD

Objective: Malignant peripheral nerve sheath tumors (MPNST) are aggressive sarcomas that occur sporadically, secondary to radiation therapy, or from malignant transformation of benign plexiform neurofibromas in individuals with the Neurofibromatosis Type 1 (NF1) cancer predisposition syndrome. MPNST are often refractory to radiation and chemotherapy with high rates of recurrence and progression to metastatic disease. Our lab previously found that TYK2 was highly expressed in the majority of MPNST and showed that Tyk2/TYK2 knockdown in murine and human MPNST cells significantly increased cell death. Furthermore, these Tyk2 knockdown cells demonstrated decreased growth capacity in subcutaneous and metastasis models. In this study, our objectives were to evaluate TYK2 expression in other soft tissue sarcomas and to determine the therapeutic potential of TYK2 inhibition alone or in combination in MPNST and other soft tissue sarcomas.

Methods: Immunohistochemistry (IHC) for TYK2 was performed on a cohort of MPNST as well as a tissue microarray composed of soft tissue sarcomas (5 MPNST, 13 leiomyosarcoma (LMS), 3 chondrosarcoma, 3 Ewing's sarcoma, 2 myxoid chondrosarcoma, and 5 angiosarcoma). MPNST and sarcoma cell lines, including LMS, angiosarcoma and synovial sarcoma, were treated with the TYK2 inhibitor deucravacitinib, alone or in combination with a MEK inhibitor (mirdametinib) in IncuCyte live cell imaging assays for cell confluence and apoptosis. IC50 values were calculated and synergy was analyzed by SynergyFinder 2.0 software. Downstream signaling consequences of TYK2 inhibition were assessed by RNA-seq, qPCR pathway array and the automated Simple Western WES system. Mirdametinib and deucravacitinib combination therapy was evaluated in multiple mouse models of MPNST in vivo.

Results: IHC revealed elevated TYK2 protein levels in 56% of MPNST, 89% of synovial sarcoma, 60% of fibrosarcoma, 54% of undifferentiated pleomorphic sarcoma and 38% of leiomyosarcoma. Following TYK2 inhibition in MPNST cells, RNA-seq and qPCR array showed upregulation of the MEK/ERK pathway and diminished expression of genes involved in the cell cycle, mitotic, and glycolysis pathways. MEK inhibitors synergized with TYK2 inhibitors to increase MPNST apoptosis and decrease cell proliferation. Interestingly, mirdametinib and deucravacitinib were also synergistically cytotoxic in other sarcoma subtypes, including SKLMS1 (non-uterine leiomyosarcoma), SKUT1 (uterine leiomyosarcoma), PCB-011 (angiosarcoma), and FUJI (synovial sarcoma) cell lines. Finally, the combination of mirdametinib and deucravacitinib dramatically reduced tumor growth in several MPNST mouse models.

Conclusion: TYK2 inhibitors decrease proliferation of MPNST cells while upregulating MEK/ERK pathway signaling in an apparent compensatory mechanism. Combining TYK2 and

MEK inhibitors synergistically blocks MPNST growth in vitro and in vivo as well as other soft tissue sarcomas in vitro. These findings support the development of a combination deucravacitinib and mirdametinib phase 1/2 clinical trial in soft tissue sarcomas with an emphasis on MPNST.

(P 275) GIST-BSF1: A NOVEL SCF-DEPENDENT, WILD-TYPE KIT CELL LINE PLATFORM THAT ALLOWS RAPID GENERATION OF ISOGENIC SUBLINES WITH BOTH IMATINIB-SENSITIVE AND IMATINIB-RESISTANT GENOTYPES

Benjamin S. Fletcher, BA[1]; Yanosan Thavarasa, BSc[2]; Susanne Grunewald, PhD[3]; Mia Purnamasari, BSc[2]; Thomas Muehlenberg, PhD[4]; Sebastian Bauer, MD[5], (1) The West German Cancer Centre, University Hospital Essen, Edinburgh, Scotland, United Kingdom, (2) Essen University Hospital, Essen, Nordrhein-Westfalen, Germany, (3) West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (4) The West German Cancer Centre, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (5) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany

Objective: Approximately 80% of Gastrointestinal Stromal Tumors (GISTs) are driven by activating mutations in the proto-oncogene KIT. These mutations result in constitutive activation of KIT, independent of the natural KIT ligand Stem Cell Factor (SCF). Most patients respond well to KIT inhibitor imatinib mesylate (IM) but the majority eventually progress due to secondary mutations in KIT. Despite a broad range of primary and secondary KIT and PDGFRA mutations observed in patients, very few primary cell lines are available for preclinical research. Here, we introduce an isogenic cell line platform allowing rapid generation of primary and consecutive mutations in KIT in a GIST cellular background.

Methods: CRISPR/Cas9-mediated gene editing was used to repair the primary KIT mutation in the well-established GIST cell line GIST-T1. Limiting dilutions fed with SCF were performed to achieve a monoclonal population with only wild-type (WT) KIT, "GIST-BSF1". KIT mutations were reintroduced to GIST-BSF1 by CRISPR/Cas9 or plasmid expression. Effects of treatment with SCF and tyrosine kinase inhibitors (TKIs) were evaluated using sulforhodamine B (SRB) cell proliferation assays and western blot.

Results: GIST-BSF1 cells show dose-dependent growth upon SCF treatment. KIT-WT signaling is stimulated by SCF and effectively abrogated by sunitinib, a known inhibitor of KIT-WT. In comparison to parental GIST-T1, GIST-BSF1 treated with SCF displayed 4.3-29.3-fold higher GR50-values for all tested KIT inhibitors, except for sunitinib (1.4-fold) and nintedanib (3.1-fold). The introduction of KIT mutations either by gene editing or plasmid led to SCF-independent growth. To date we have established a panel of 20 cell lines harboring different primary, secondary and tertiary KIT mutations. Evaluation of a panel of approved and non-approved KIT inhibitors showed inhibitory profiles expected for the respective mutations. GIST-BSF1 sublines with secondary mutations in-cis in both the ATP binding pocket (AP) and activation loop (AL) of KIT, called AP/AL mutations, display the same inhibitory profile towards ripretinib as seen in AP/AL mutations previously modelled in GIST-T1 (GR50 values for AP/AL mutants >20-fold higher than corresponding AL mutants). Parallel gene knock-out of NF1 and PTEN as well as introduction of activating mutations in PIK3CA result in SCF-independent cell growth and confer resistance to TKIs targeting KIT.

Conclusion: GIST-BSF1 is the first KIT-WT, SCF-dependent GIST cell line, which allows study of the impact of KIT inhibitors on WT KIT. Reintroduction of KIT mutations leads to SCF-independent growth and yielded anticipated TKI profiles. This cell line platform allows the rapid validation of novel variants of KIT and other oncogenic kinases in a GIST-specific cellular background.

(P 276) REVISITING IN- AND OFF LABEL DRUGS FREQUENTLY USED IN METASTATIC GIST USING A BROAD IN VITRO PROFILING PANEL OF PRIMARY AND SECONDARY KIT AND PDGFRA-MUTANT GIST CELLS – CLINICAL IMPLICATIONS

Thomas Muehlenberg, PhD[1]; Benjamin S. Fletcher, BA[2]; Dawid Krzeciesa, M.Sc.[1]; Rainer Hamacher, MD[3]; Valerie Haller[4]; Yasmin Zaun, MD[4]; Moritz Kaths, MD, MSc[4]; Jürgen Treckmann, MD[5]; Johanna Falkenhorst, MD[3]; Sebastian Bauer, MD[3], (1) The West German Cancer Centre, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (2) The West German Cancer Centre, University Hospital Essen, Edinburgh, Scotland, United Kingdom, (3) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (4) University Hospital Essen, Sarcoma Centre, Essen, Nordrhein-Westfalen, Germany, (5) University Hospital Essen, Essen, Nordrhein-Westfalen, Germany

Objective: Resistance to imatinib in KIT-mutant and avapritinib in PDGFRA-mutant GIST is mostly driven by secondary mutations of KIT or PDGFRA in metastatic gastrointestinal stromal tumors (GIST). Despite five approved kinase inhibitors for the treatment of GIST, most patients eventually progress and tyrosine-kinase inhibitors not approved for GIST are frequently used in an off-label setting based on small trials in the pre-regorafenib and/or ripretinib era. Here, we used our novel and comprehensive panel of cell lines covering primary and secondary resistance mutations to identify genotypes that would benefit from off-label treatment.

Methods: Cas9-based gene editing was used to generate a panel of isogenic cell lines comprising 16 KIT- and 5 PDGFRA-mutant sublines. Sulforhodamine B (SRB) assays were used to study cytotoxicity/proliferation. Apart from approved drugs (imatinib, sunitinib, regorafenib, ripretinib, and avapritinib, we evaluated cabozantinib, pazopanib, dasatinib, and sorafenib. Immunoblotting was used to evaluate effects on KIT and KIT dependent signaling.

Results: Secondary mutations within the ATP-binding pocket (AP) and the activation loop (AL) confer high levels of resistance to IM (GR50: 30- to >500-fold higher than exon 11 primary mutation) with the exception of the D820Y mutation (5-fold). Pazopanib exhibits no activity (GR50: >5uM) in any resistant subline with the exception of the C809G secondary resistance mutation, which is effectively inhibited by ripretinib and sunitinib. Cabozantinib is highly effective against primary exon 11 and exon 9 mutations and the T670I secondary mutation but shows a 100-fold higher GR50 against the common AP mutation V654 and >50-fold higher GR50 against most AL mutations (except D820Y). Dasatinib is highly potent against primary mutations (GR50: 1-6nM) and AL-mutations C809G (GR50: 1nM) and D820Y (GR50: 3nM) but shows only modest activity against other AL-mutations (33-94nM). Sorafenib exhibits a highly similar inhibitory profile compared to regorafenib. PDGFRA-D842V mutant GIST are potently inhibited by avapritinib only. No activity is seen with any drug against D842V-sublines harboring secondary resistance mutations.

Conclusion: Preclinical testing suggests that dose-escalation of or retreatment with imatinib is

not effective in exon 11 mutant GIST with secondary mutations. Pazopanib appears highly ineffective against any secondary mutation but shows notable activity in exon 9 mutant GIST. Cabozantinib is the only drug with strong activity against the gatekeeper T670I and the AL D820Y mutation. Avapritinib but no other drug shows activity in PDGFRA-D842V mutant GIST. Comprehensive preclinical profiling does not identify a particular genomic niche for off-label drugs. However, in an era of sensitive ct-DNA testing this data could be useful in the context of un-manageable toxicity or in health-systems with lack of access to some of the approved drugs.

(P 277) **ANTI-GD2 SYNERGIZES WITH HYPOXIA PATHWAY TARGETING TO BLOCK OSTEOSARCOMA CELL PROLIFERATION AND MIGRATION.**

Natacha ENTZ-WERLE, MD, PhD[1]; Marina Pierrevelcin, PhD[2]; Romain Vauchelles, ENGINEER[3]; Benoit Lhermitte, MD[4]; Eric Guerin, PharmD, PhD[4]; Françoise Françoise, PhD[5]; Andreas Reisch, PhD[3]; Andrey Klimchenko, PhD[3]; Vincent Flacher, PhD[6]; Nathalie Gaspar, PhD MD[7]; Charlotte Nazon, MD[8]; Monique Dontenwill, PhD[3], (1) University hospital of Strasbourg and UMR CNRS 7021, Strasbourg, Alsace, France, (2) UMR CNRS 7021, strasbourg, Alsace, France, (3) UMR CNRS 7021, Strasbourg, Alsace, France, (4) University Hospital of Strasbourg and UMR CNRS 7021, Strasbourg, Alsace, France, (5) UMR_S 1307 Center for Research in Cancer and Integrated Immunology, Nantes, Pays de la Loire, France, (6) IBMC, Strasbourg, Alsace, France, (7) Gustave Roussy cancer campus, Paris, Ile-de-France, France, (8) University Hospital of Strasbourg, Strasbourg, Alsace, France

Objective: Osteosarcoma (OTS), the most common primary malignant bone tumor in pediatrics, remains a challenge to treat and cure, especially in case of highly resistant disease. Among the list of extra-tumoral mechanisms enabling the cancer cell resistance, hypoxia and tumor-associated macrophage modulation seem to be in the top ranks. Here, we aim to study hypoxic features in OTS tumors and in innovative in vitro OTS patient-derived models focusing on mTor and HIF-1α as major actors of the hypoxic environment additionally to GD2 overexpression. The ganglioside GD2, known to be a key marker of mesenchymal/osteoblast precursor and a cell surface–associated antigen, is serving as a target of macrophage immunotherapy. So, our objectives were to confirm first the expression of all those biomarkers (e.g., mTor, HIF-1 and GD2) in OTS tumors and models. Thereafter, we evaluate efficacy of rapamycin, irinotecan and dinutuximab beta in single drug approach in each model and proceed to combination assessments to understand their impact on OTS cell proliferation and migration and enable the selection the potential therapeutic combo to use.

Methods: Using in vitro OTS patient-derived models (cell lines and tumoroids), patient-derived xenografts (PDX), tumoroids combining patient-OTS cells and macrophages and tumors' collection, we, first, determined by CGHarray, immunohistochemistry, immunofluorescence, western blot analysis and quantitative PCR the presence of all hypoxia and GD2 biomarkers. Thereafter, based on proliferation and migration assays, we proceed to drug screening using mTor, HIF1 and GD2 targeting in single or combo approaches. Finally, using PDX, we confirmed the combo approach.

Results: First, we were able to confirm the involvement of mTor, HIF1 and GD2 hyperexpressions in OTS cells and tumors. Using the several models (patiet-derived cell lines, tumoroids and PDX) and combining blockade of those targets, we evidenced in our in vitro OTS models using proliferation assay, western blotting, immunofluorescence, migration experiments a synergism between mTorc1 and HIF-1α inhibitors (e.g., rapamycin and irinotecan,

respectively) and when associating anti-GD2 (Dinutuximab Beta). Those data provide the rationale for developing combination therapeutic strategies in resistant OTS. The combo were validated in animal model.

Conclusion: Clearly, we were able to show in patient biopsies, as well as in our patient-derived models that the 3 targets were largely expressed and might be efficiently inhibited to prevent OTS cell proliferation and migration.

(P 278) CLINICAL MARKERS PREDICTIVE OF TRABECTEDIN RESPONSE IN SOFT TISSUE SARCOMA

Charlotte vH Widlund[1]; Andri Papakonstantinou, MD, Ph.D[2]; Ioannis Zerdes, MD, Ph.D[2]; Subhash Srivastava, MD, Ph.D Candidate[2]; Christina L. Stragliotto, MD, Ph.D[3]; Panagiotis Tsagkozis, MD, Ph.D[2]; Felix Haglund de Flon, MD, Ph.D[2], (1) Karolinska University Hospital, Lidingö, Stockholms Lan, Sweden, (2) Karolinska University Hospital, Stockholm, Stockholms Lan, Sweden, (3) Karolinska University Hospital, Spånga, Stockholms Lan, Sweden

Objective: To identify factors predictive of trabectedin response in patients with metastatic soft tissue sarcoma.

Methods: A total number of 93 patients treated with trabectedin at the Karolinska University Hospital Sarcoma Centre between 2012 and 2020 were identified retrospectively. Eligibility criteria for inclusion in the study included age over 18 years and that the patients received at least one cycle of trabectedin. Available patient, biochemical and tumor-related data from medical records were collected. Primary end points were progression-free survival (PFS), defined as the time from the first administration of trabectedin to the date of documented disease progression or death and overall survival (OS), defined as the time from the first administration of trabectedin to the date of death from any cause. Descriptive statistics was performed for the entire study population and Kaplan-Meier and the log-rank test was used for determination of statistical differences in survival curves between groups. Cox regression analyses were performed to evaluate the independent effects of the candidate variables on PFS and OS respectively. All p-values were 2-sided and the significance level set to $\alpha=0.05$. As this was an explorative study, no adjustment for multiple testing has been performed. All statistical analyses were performed with IBM SPSS Statistics 25 software.

Results: Clinical outcomes
The median PFS and OS in the entire study population was 4.4 months (95% confidence interval (CI) 2.3-6.6) and 16.2 months (95% CI 12.3-20.1) respectively.

Predictive factors of PFS and OS
Five of the explored variables were significantly associated ($p < 0.05$) with either PFS and/or OS; histological subtype, hemoglobin, albumin, white blood cell count (WBCC) and absolute neutrophil count (ANC).

Progression-free survival
In the univariable analysis of PFS, three of the variables were significantly ($p < 0.05$) associated with PFS; albumin, WBCC and ANC.

Higher baseline albumin level was associated with better PFS (Hazard Ratio [HR]=0.95; 95%

CI 0.90-1.00, p=0.03) whereas higher level of WBCC as well as higher level of ANC were associated with worse PFS (HR=1.05; 95% CI 1.00-1.10, p=0.04) and (HR=1.06; 95% CI 1.01-1.11, p=0.01) respectively.

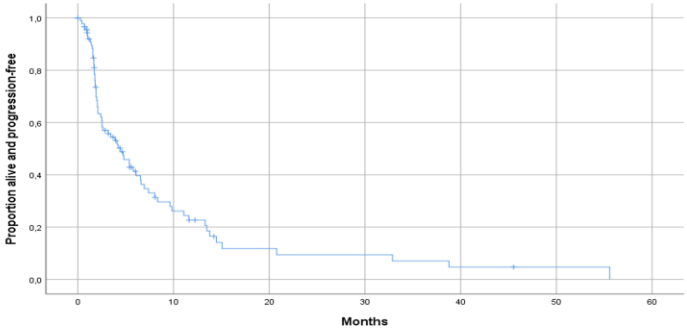
Median PFS for albumin < 35 (g/L) versus albumin ≥35 (g/L) was 2.5 months (95% CI 0.8-4.2) versus 5.8 months (95% CI 3.3-8.3) (log rank test, p=0.02). Median PFS for ANC ≤7.5 (10^9 cells/L) versus ANC >7.5 (10^9 cells/L) was 5.4 months (95% CI 3.1-7.7) versus 1.8 months (95% CI 1.6-2.0) (log rank test, p=0.02).

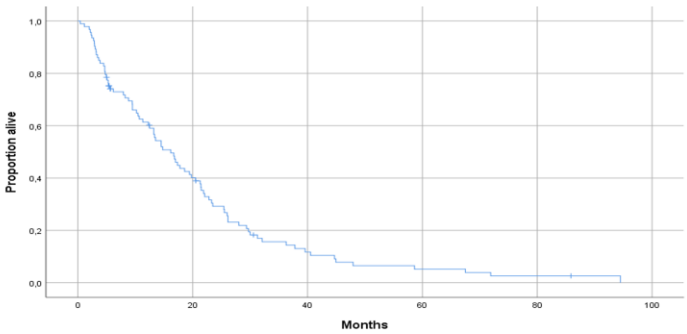
Overall survival
In the univariable analysis of OS, the same baseline biochemical factors as in PFS analysis were associated with OS, but with the addition of hemoglobin also being associated. Furthermore, histological subtype was associated with OS.

Other histological subtype versus L-sarcoma was associated with worse OS (HR=1.79; 95% CI 1.09-2.95, p=0.02). Higher baseline level of hemoglobin was associated with better OS (HR=0.98; 95% CI 0.97-1.00, p=0.04).

Median OS for histological subtype other versus histological subtype L-sarcoma was 12.5 months (95% CI 6.9-18.0) versus 17.8 months (95% CI 13.1-22.5).

Conclusion: The results from this study support previously published results of L-sarcoma being associated with better outcome (OS) of trabectedin compared to other histological subtypes in patients with metastatic soft-tissue sarcoma. Moreover, we demonstrate that the baseline biochemical factors hemoglobin, albumin, ANC and WBCC can predict response to trabectedin in patients with metastatic soft-tissue sarcoma in terms of PFS and OS. There are hopeful indications on easily collected biochemical baseline data having the potential to serve as decision support for the selection of patients in favor of this treatment.





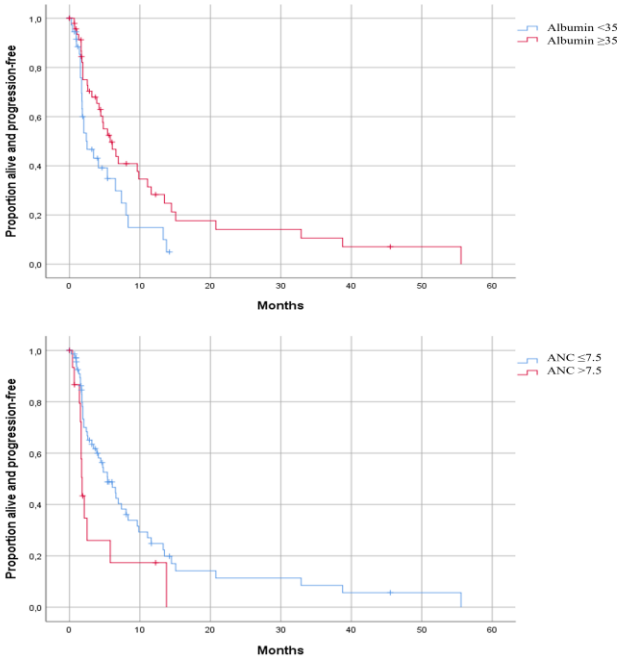
Clinical outcomes	n (%)	Median (95% CI)
PFS analysis (n=93)		
Events	66 (71)	
Censored	27 (29)	
PFS (months), CI		4.4 (2.3-6.6)
OS analysis (n=93)		
Events	84 (90)	
Censored	9 (10)	
OS (months), CI		16.2 (12.3-20.1)
Reason for cessation of trabectedin treatment (n=90)		
Disease progress	64 (71)	
Adverse event/toxicity	20 (22)	
Other	4 (4)	
Death	2 (2)	

Variable		PFS		OS	
		HR (95% CI)	*p*	HR (95% CI)	*p*
Age		1.01 (0.99-1.03)	0.48	1.01 (0.99-1.03)	0.16
Characteristics of primary tumor					
Histological subtype					
L-sarcoma	ref	1		1	
Other		1.50 (0.86-2.64)	0.16	**1.79 (1.09-2.95)**	**0.02**
Grade					
Low or intermediate	ref	1		1	
High		1.16 (0.39-3.47)	0.79	0.53 (0.21-1.33)	0.18
Location					
Abdomen	ref	1		1	
Trunk		1.09 (0.46-2.60)	0.85	1.95 (0.97-3.90)	0.06
Extremities		0.60 (0.29-1.24)	0.17	0.96 (0.51-1.81)	0.89
Other		1.60 (0.71-3.61)	0.26	1.96 (0.88-4.38)	0.1
Baseline biochemical data					
Hemoglobin		0.99 (0.98-1.01)	0.48	**0.98 (0.97-1.00)*** **	**0.04**
Albumin		**0.95 (0.90-1.00)***	**0.03**	**0.90 (0.86-0.94)**	**0,00**
LD		1.02 (0.89-1.18)	0.8	1.08 (0.98-1.19)	0.12
WBCC		**1.05 (1.00-1.10)****	**0.04**	**1.06 (1.02-1.10)**	**0,00**
ANC		**1.06 (1.01-1.11)**	**0.01**	**1.07 (1.03-1.11)**	**0,00**
Platelet count		1.00 (1.00-1.00)	1,00	1.00 (1.00-1.00)	0.18

*95 % CI (0.901-0.995)

**95 % CI (1.002-1.099)

***95 % CI (0.965-0.999)



(P 279) **PROTEIN PHOSPHATASE 2 A INHIBITION AS A MECHANISM TO ENHANCE DOXORUBICIN ACTIVITY**

Marta Martín-Ruiz, PhD[1]; David S. Moura, PhD[2]; Marta Renshaw, Technician lab[2]; Elena Blanco Alcaina, Master degree[3]; Jose L. Mondaza-Hernandez, Master degree[2]; Pablo Romero, Master degree[2]; Jaime Carrillo, PhD[2]; Davide Di Lernia, MD[2]; Nadia Hindi, MD, MSc[4]; Javier Martín-Broto, MD, PhD[2], (1) Instituto de Investigaciones Sanitarias Fundación Jiménez Díaz, Madrid, Madrid, Spain, (2) Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM),, Madrid, Madrid, Spain, (3) Institute of Biomedicine of Sevilla, IBIS, Hospital Universitario Virgen del Rocío-HUVR, Consejo Superior de Investigaciones Científicas-CSIC, Sevilla, Andalucia, Spain, (4) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain

Objective: Heterogeneity of sarcoma renders challenging the development of new therapeutic strategies. New options are needed, moreover in patients with advanced soft-tissue sarcoma (STS). Doxorubicin (D) is still the standard of first line in advanced STS, and several drug combinations have failed to demonstrate a survival benefit over doxorubicin alone, in phase III trials. Protein Phosphatase 2A (PP2A) is a protein involved in DNA damage repair mechanisms. LB-100, a PP2A inhibitor (PP2Ai), has been described to impair DNA damage repair mechanisms, synergizing with chemotherapy or radiotherapy, especially in cancer cells. We hypothesize that LB100 will synergize with doxorubicin, enhancing its activity and increasing cell death in preclinical models of STS. Our objetives are: - To determine the potential synergy between LB-100 and doxorubicin in in vitro studies. - To evaluate the activity of LB-100 plus doxorubicin in patient-derived xenograft (PDX) models of leiomyosarcoma (LMS) and undifferentiated pleomorphic sarcoma (UPS). - To assess the mechanisms

underlying the activity of this combination in both in vitro and in vivo studies.

Methods: STS human cell lines of leiomyosarcoma (AA, CP0024, SK-UT-1 and IEC005) and leiomyosarcoma PDX model, were treated with D, LB100 and D + LB100. Cell viability was assessed by MTS after 72 hours. Based on synergy results, the best treatment sequence was selected for functional studies. Briefly, the effect of D + LB-100 tested in: apoptotic cell death, by western blot (WB) (i.e. cleaved PARP1, cleaved caspase-3) and flow cytometry. DNA damage accumulation, by WB and immunofluorescence (e.g. ATR, ATM, γH2AX, RAD51, RPA foci accumulation). Statistical analysis was evaluated by MTS assay, and IC-50 was determined by fitting a nonlinear regression analysis to each model.

Results: Significant differences between D monotherapy and D + LB-100, based on IC-50 cell viability were found. The increase in apoptosis of all cell lines analysed could be related with PP2A function in the DNA. Two mechanisms were involved, the homologous recombination (HR) and non-homologous end joining (NHEJ). Both repair pathways are active; however, the NHEJ pathway (53BP1...) was generally less efficient than the HR pathway (ATR, ATM, RAD51, RPA yH2AX...) in the absence of a homologous template.

Conclusion: PP2A is involved in the doxorubicin mechanism and LB-100 is a good enhancer of these treatments in STS (at least in leiomyosarcoma). This study opens a new insight to further understand the mechanisms in different sarcoma subtypes, for the translational studies to the clinical patients. A comprehensive analysis of the mechanisms underlying sensitivity or resistance to doxorubicin will generate new research hypothesis for future preclinical and clinical studies.

(P 280) A CELL-BASED COMPOUND SCREENING IDENTIFIES AGENTS THAT SYNERGIZE WITH EVEROLIMUS IN SARCOMA

Zhenfeng Duan, MD, PhD[1]; Robert L. Walker, BS[2]; Jonathan Trent, MD, PhD[3]; Francis Hornicek, MD, Ph.D[1]; Gina Z. D'Amato, MD[4], (1) University of Miami, Miami, FL, (2) University of miami, Miami, FL, (3) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, (4) Sylvester Comprehensive Cancer Center, Miami, FL

Objective: Everolimus, an inhibitor of the protein kinase mTOR, has shown preclinical and clinical efficacy in its ability to inhibit tumor growth. However, there has been limited success in selectively targeting mTOR alone. The aim of the current study is to find potent targets that a have synergistic effect with everolimus in sarcoma therapy.

Methods: To search for new chemical entities to enhance the efficacy of mTOR targeting by everolimus in sarcoma, a preselected drug-like small molecular compound library of kinase inhibitors was initially screened using osteosarcoma cell lines and further evaluated in chondrosarcoma and chordoma cell lines. To examine the effectiveness and underlying mechanisms behind the identified synergistic agents, several experiments were performed, including MTT and clonogenic assays as well as Western blot.

Results: The screen identified dasatinib, a Src inhibitor, as having the most potent synergism with everolimus. The results confirmed that everolimus and dasatinib have a potent synergistic effect as significant reductions in cell viability were observed as well as the mitigation of the overexpression of key signaling pathway downstream targets. Everolimus and dasatinib induce

effective synergism that reduces osteosarcoma cell proliferation and elicits apoptosis. This synergistic effect is likely due to the inhibition of mTOR by everolimus which potentially facilitates dasatinib-induced Src deactivation. Dasatinib-induced Src inhibition further suppresses the activation of mTOR via the PI3K-AKT signaling pathway.

Conclusion: Our work shows targeting mTOR and the Src signaling pathways via combinatory treatment is a potentially effective therapeutic strategy for the treatment of osteosarcoma. Since everolimus and dasatinib are already in phase I/II clinical trials, this knowledge could soon be translated into the clinic.

(P 281) FIVE DAY SPACING OF TWO FRACTIONATED ABLATIVE RADIOTHERAPIES ENHANCES ANTITUMOR IMMUNITY IN FIBROSARCOMA CELL LINE

Mi-Sook Kim, PhD[1]; hyunkyung Kim, PhD[2]; Eunju Lee, MS[2]; Haeun Cho, BS[2]; Eunji Kim, MD[2]; Won Il Jang, MD, PhD[2]; Kwangmo Yang, MD[2]; Yoon-Jin Lee, PhD[2]; Tae-Jin Kim, PhD[2], (1) Korea Cancer Center Hospital, Seoul, Seoul-t'ukpyolsi, Republic of Korea, (2) Korea Institute of Radiation and Medical Sciences, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Objective: This study aimed to enhance tumor control and abscopal effects by diverse stereotactic ablative body radiation (SABR) schedules.

Methods: FSaII (fibrosarcoma) cell was used for tumor growth delay assays after 1- or 5-day interval radiotherapy (RT) with 40 Gy. Immunodeficient BALB/c-nude, immunocompetent C3H mouse models were used. For immune monitoring, FSaII tumors were analyzed using flow cytometry, immunofluorescence staining, and qRT-PCR. The spleens were used for the ELISpot assay and flow cytometry to determine effector CD8 T-cells. Micrometastatic lung lesions were analyzed for the abscopal effect.

Results: Contrary to the high-dose 1-day interval RT, the 5-day interval RT significantly suppressed tumor growth in immunocompetent mice; this was not observed in immunodeficient mice. Five-day spacing was more effective than 1-day interval in enhancing the anti-tumor immunity via increasing the secretion of tumor-specific IFN-γ, activating the CD8 T-cells, and suppressing the monocytic myeloid-derived suppressor cells (M-MDSCs). The 5-day spacing inhibited lung micrometastases more effectively than did 1-day interval in FSaII.

Conclusion: Compared to the one-day interval RT, the five-day interval RT scheme demonstrated higher CD8 T-cell activity through M-MDSC inhibition. High levels of anti-tumor immunity resulted in high levels of tumor control indicating that FSaII tumors as a hot tumor could trigger a strong anti-tumor immune response. These results point to a novel therapeutic method for improving local clinical control during SABR: altering the time gap between fractions. In addition, FSaII tumors showed an abscopal influence on lung metastasis. To significantly boost an abscopal effect in various tumors, which are immunologically hot and cold, increased immune activation with ideal SABR fraction intervals would be required.

(P 282) AN EMBRYONIC ZEBRAFISH XENOGRAFT MODEL OF RHABDOMYOSARCOMA REVEALS GENOTYPE DEPENDENT TUMOUR VASCULARISATION DYNAMICS AND RESPONSE TO ANTI-ANGIOGENIC RECEPTOR TYROSINE KINASE INHIBITORS

Joseph W. Wragg, PhD; Emma Gray, MRes; Jo Morris, PhD; Andrew Beggs, FRCS (Gen), PhD; Ferenc Mueller, PhD; Susanne A. Gatz, MD/PhD, University of Birmingham, Birmingham, England, United Kingdom

Objective: Rhabdomyosarcoma (RMS) is a highly aggressive soft tissue sarcoma, predominantly affecting children and adolescents. Treatment includes intensive chemotherapy, surgery and radiotherapy and outcome has remained largely unchanged over the last decades with 5-year survival still < 30% for some cohorts of patients. Better therapeutic regimens including novel agents are urgently needed. Multi receptor tyrosine kinase inhibitors (MRTKIs) are an important class of agents, many of which are licensed for adult indications and explored in the clinic in sarcoma patients including children and young adults. The MRTKI Regorafenib is currently assessed in the relapse setting in patients with RMS in the Frontline and Relapsed RhabdoMyoSarcoma (FaR-RMS) trial (NCT04625907). Strikingly, biomarkers of response for these agents have yet to be identified. MRTKIs act against not only the cancer cell, but also the supporting stroma, particularly the vasculature. This interaction is poorly modelled in standard cell culture and in vivo mouse xenograft studies. The early embryonic zebrafish is translucent and provides an opportunity to assess this interaction with high throughput in vivo imaging. We have, therefore, developed a novel embryonic zebrafish xenograft model of RMS where we can directly visualise tumour and vasculature development and assess the effects of MRTKIs.

Methods: FLK-1-GFP (blood vessel reporter) transgenic zebrafish embryos were inoculated in the yolk with fluorescently labelled cells from 8 different RMS cell lines (fusion-positive (FP): Rh4, Rh30, Rh41, RMS-01, fusion-negative (FN): RD, JR1, CT10) and a healthy control line (HFF1)), at 2-day post-fertilization and incubated at 34C for up to 3 days. Tumour and vessel development were assessed using a Cytation-5 microscope. Images were analysed using custom FIJI pipelines to generate multifaceted metrics of tumour development and vascularisation. Tumour growth assessments were validated through cell counting. Three clinically relevant MRTKIs (Regorafenib, Infigratinib and Axitinib) with different target receptor profiles were used in this study. To identify the appropriate treatment dose, non-tumour bearing embryo zebrafish were exposed to a range of drug doses (0-10μM) to identify the highest dose where no overt side effects on embryo development are observed.

Results: All 7 RMS cell lines engrafted in the embryo zebrafish with a tumour take rate of >90%, compared with 0% for HFF1 control cells. Tumour burden assessment by fluorescent imaging indicated growth during the observation period for all models. Direct cell counts, performed for RD and Rh4, correlated with imaging data (r=0.85) and confirmed high viability (RD = 86.7%; Rh4 = 84.1%). Inoculated RMS tumours induced both, neo-vascularisation towards the tumour (60%-98% of cases) and increased density of proximal vessel beds. Correlation analysis revealed RMS subtype specific differences in vessel induction, with neo-vascularisation correlating with tumour burden in FN lines (r=0.58-0.8) and proximal vessel bed complexity correlating with tumour burden in FP lines (r=0.43-0.61). Tumour and associated vessel treatment responses were assessed for the MRTKis Regorafenib, Infigratinib and Axitinib at the maximum tolerated doses of 0.1 μM, 0.1 μM and 0.05 μM, respectively. This revealed significant heterogeneity in treatment response, but also primary site of drug action. This was most apparent between FN RD and FP Rh4 cells in response to Infigratinib, where the drug achieved an 80% reduction in Rh4 tumour burden compared to 35% in RD, but only a 65% reduction in neo-vessel induction compared to 90% in RD. The observation of inter tumour

cell differences in tumour growth versus vascularisation inhibition suggests additional value of our model for response prediction to MRTKIs.

Conclusion: We have developed a novel RMS xenograft model, utilising the unique imaging properties of the embryonic zebrafish to profile the initial tumour-stroma interactions in developing RMS tumours. This model allows assessment of tumour growth, vascularisation initiation and therapeutic responses to clinically relevant MRTKIs. Significant heterogeneity was observed and correlative analysis with the presence of autocrine signalling factors and expression of receptor tyrosine kinases on RMS cells is ongoing. Future work will include the assessment of patient-derived tumour cells (ideally from ongoing clinical trials), alongside comparative analysis with standard preclinical models to define the value of this model in preclinical drug development and identify biomarkers of response to MRTKIs.

(P 283) A TRIDIMENSIONAL PRECLINICAL MODEL TO STUDY HUMAN AND CANINE OSTEOSARCOMA DEVELOPMENT AND INTERACTION WITH TUMOR MICROENVIRONMENT

Ania Naila Guerrieri, M. Sc, PhD[1]; Gabriele Scattini, M. Sc, PhD[2]; Elia Bari, M. Sc, PhD[3]; Andrea Cacciamali, M. Sc.[4]; Martina Angela Checco, M. Sc.[4]; Aurora De Mattia, M. Sc.[4]; Stefania Benini, M. Sc.[5]; Marco Gambarotti, MD[6]; Chiara Bellotti, M. Sc.[1]; Micaela Pannella, M.Sc, PhD[1]; Luisa Pascucci, DVM, PhD[2]; Maria Luisa Torre, M. Sc, PhD[7]; Toni Ibrahim, BSc, MSc, MD, PhD[1]; Silvia Dotti, DVM, PhD[8]; Enrico Lucarelli, M. Sc, PhD[1], (1) Istituto Ortopedico Rizzoli IRCCS, Osteoncology- Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (2) Department of Veterinary Medicine, University of Perugia, Perugia, Umbria, Italy, (3) University of Eastern Piedmont - Department of Pharmaceutical Sciences, Novara, Piemonte, Italy, (4) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Laboratorio di Controllo di Prodotti Biologici, Centro di Referenza Nazionale per i Metodi Alternativi, Benessere e Cura degli Animali da Laboratorio, Brescia, Lombardia, Italy, (5) Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Emilia-Romagna, Italy, (6) Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (7) Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Piemonte, Italy, (8) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Laboratorio di Controllo di Prodotti Biologici, Centro di Referenza Nazionale per i Metodi Alternativi, Benessere e Cura degli Animali da Laboratorio, 25124 Brescia, Brescia, Lombardia, Italy

Objective: Osteosarcoma (OS) is a rare primary bone tumor affecting humans and pets, particularly large bread dogs, that develops mainly in the medullary cavity of the axial skeleton. OS tumor microenvironment is characterized by the formation of woven bone with defective biomineralization, and its rapid growth may cause osteolytic lesions, which can free growth factors stored in the bone matrix and undermine bone strength. The gold standard treatment in both species resides in chemotherapy and surgery, which are not curative in all cases, entailing the risk of developing a local recurrence or distant metastases. Animal models still represent the optimum for pathology and pharmacological studies, but the Italian legislation has placed special attention on developing alternative models of laboratory animals. Our aim is to develop a 3D in vitro model of human and canine OS by culturing cancer cells encased within alginate-based capsules in the presence or absence of demineralized bone matrix (DBM), which simulates the matrix in osteolytic lesions and woven bone. Our goals are to assess the ability of

the model to "recreate" a similar tissue to that of origin and to evaluate the influence of part of the tumor microenvironment, such as the DBM. Finally, we aim to highlight differences or similarities between human and dog cancer cells.

Methods: Alginate capsules were produced by dripping a mixture of cells in culture medium, xanthan gum, and CaCl2 with/without DBM in an alginate solution in agitation. The cell lines were 143B-GL (aggressive metastatic, +GFP/Luciferase) and MG63-GL (fibroblastic non-metastatic, +GFP/Luciferase) for the human model, and D17 cells for canine OS. Capsules were cultivated for 21 days in static conditions. On days 1, 7, 14, and 21, we performed cellular viability, gene expression, immunohistochemical (SATB2, alcian blue, alizarin red staining), and fluorescence/confocal microscopy analyses.

Results: All cell lines display a sustained growth inside the capsules for at least 14 days, forming 3D aggregates of different sizes. Between day 14 and day 21, while human OS cells enter growth arrest, canine D17 cells continue proliferating. The 143B-GL cells show a slight surge in proliferation in the presence of DBM, while MG63-GL and D-17 cells have no effects. All cell lines tend to grow around the DBM. Interestingly, in human OS models, the analysis of cell viability at day 21 displays a significant higher number of MG63-GL cells evading the capsules in the presence of DBM, reaching the potential of the invasive 143B-GL cell line. Both fluorescence and confocal microscopy analyses show a structural change of DBM in all samples, suggesting that cancer cells could remodel the matrix. Histochemical staining reveal that from day 7, all cells deposit calcium and collagen, indicating moderate osteoinductive properties and initial mineralization. This is more evident for MG63-GL cells, which are defined as osteoblast-like cells. In all samples, the addition of DBM induces a major calcium deposition, indicating a good reproduction of the tumor microenvironment. Finally, the positivity to SATB2 confirms the consistency of our model after 21 days of culture.

Conclusion: Our 3D models demonstrated good potential to recreate the human and canine OS microenvironment in vitro. The presence of DBM not only influenced the cells' way of growing, but the results on the MG63-GL cell line suggest that cells that are known in the literature to represent an indolent form of OS acquire the ability of motility in the presence of a component that mimics tumor microenvironment. Further analyses are needed to better define the accuracy and reliability of our model with respect to the in vivo tumors. Once optimized, our model will allow to test various drugs, such as antiproliferative chemotherapeutics from 7 to 14 days of culture, when cells are still in their exponential growth phase, and pro-differentiating drugs to study and induce OS cell osteo-differentiation.

(P 284) VALIDATING A NOVEL MICROFLUIDIC PLATFORM AS AN EX VIVO TUMOUR MODEL FOR SARCOMA DRUG DISCOVERY

Kousha Kamal, BSc[1]; Philip Wong, MD[2]; Kim Tsoi, MD, PhD[1], (1) University of Toronto, Toronto, ON, Canada, (2) Princess Margaret Cancer Centre, Toronto, ON, Canada

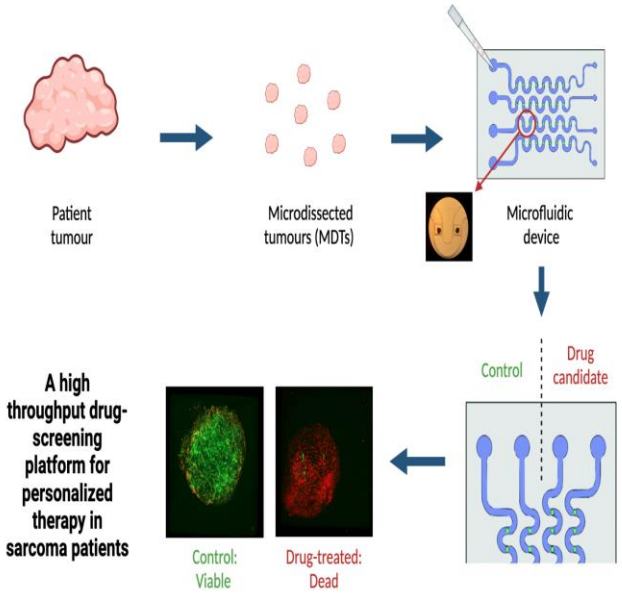
Objective: BACKGROUND:
Sarcomas are a rare and heterogeneous group of mesenchymal cancers characterized by more than 70 distinct subtypes. In the near 25–50% of sarcoma patients that develop metastatic disease, the median survival is around 15–20 months, in part due to a low response rate to traditional systemic therapies. More recently, the immune microenvironments of various

sarcoma subtypes have been identified as an important factor in their growth and metastatic activity, presenting researchers with new potential targets for therapeutic drugs. However, the current cancer drug development pipeline is hindered by the limitations of preclinical models to correctly predict clinical outcomes in patients. This is partly due to the inability of cell cultures and mouse models to fully recapitulate the microenvironment of human tumours. Additionally, due to high patient-to-patient variability of sarcoma diagnoses, there is a desperate need for a new patient-specific drug-testing tool to guide the choice of therapy in the clinic. A potential solution to this problem is a tumour-on-chip platform that uses microfluidics to culture sub-millimetre micro-dissections of patient-derived tumours (MDTs). Unlike other ex vivo tissue culture methods, this microfluidic platform can keep MDTs viable for up to 15 days, which is a suitable timeline for most drug-testing assays. Furthermore, each device can house up to 32 MDTs in four different channels, allowing for high-throughput parallel testing of different treatment conditions within the same device. So far, this platform has been tested on several different cancer types, but has yet to be validated for soft-tissue sarcoma. At Mount Sinai Hospital, our research team has access to the largest sarcoma reference in Canada, with surgically resected tumours being available to us on a weekly basis. As a result, we are in a unique position to validate this platform for a variety of sarcoma subtypes.

Methods: HYPOTHESIS AND OBJECTIVES:
It is hypothesized that the MDT-on-chip device is a representative ex vivo model of sarcoma tumours. To evaluate this hypothesis, we will: 1) assess the viability and genetic composition of sarcoma MDTs within the device over time; 2) examine the ability of MDTs to recapitulate the immune microenvironment of the source tumour.

Results: ONGOING AND UPCOMING EXPERIMENTS:
1) ONGOING: to examine how long sarcoma MDTs can be cultured in the device, samples will be stained using live/dead fluorescent dyes and imaged via confocal microscopy at several time-points over a 15-day period. The resulting z-stack images will then be analyzed to quantify the ratio of live-stained cells over total stained cells, which will serve as a measure of viability. Furthermore, whole-exome sequencing will be used to measure changes in the genomic composition of MDTs over time with respect to the source tumour. This analysis will identify how long the MDTs can be cultured in the device before experiencing genetic drift. 2) UPCOMING: an immune profile of the MDTs will be defined using immunohistochemistry and immune cell deconvolution analysis of RNA sequencing data. The relative abundance of T cells, B cells, natural killer cells, dendritic cells and macrophages will be compared to that of the source tumour to examine the ability of MDTs to recapitulate its immune microenvironment. An emphasis will be placed on tumour-associated macrophages (TAMs), as they have been identified as a major immune component of various sarcoma subtypes, and are a target-of-interest for immunotherapy development in our lab. To that effect, a functional assay will be performed on the MDTs to evaluate the phenotype of TAMs in response to stimulation at various time-points within the device.

Conclusion: SIGNIFICANCE OF STUDY:
If proven to be a representative model of the patient tumour, the MDT-on-chip platform could greatly benefit the drug discovery pipeline of sarcoma by offering a high throughput method for personalized testing in both the lab and the clinic.



(P 285) TUMOUR-INFILTRATING MAST CELLS IN ANGIOSARCOMAS CORRELATE WITH CLINCIAL PHENOTYPES AND IMMUNO-ONCOLOGY PATHWAYS

Elizabeth Chun Yong Lee, Bsc[1]; Boon Yee Lim, N/A, n/a[1]; Bavani Kannan, N/A, n/a[2]; Jing Yi Lee, n/a[2]; Tun Kiat Ko[2]; Cedric Chuan-Young Ng, N/A, n/a[2]; Bin Tean Teh, N/A, n/a[1]; Jason Yongsheng Y. Chan, MBBS, MRCP, MMED, PhD[2]; Sarah Beishan B. Tai, MBBS, Bsc, MMed, MRCP[1], (1) National Cancer Center Singapore, Singapore, N/A, Singapore, (2) National Cancer Centre Singapore, Singapore, N/A, Singapore

Objective: Angiosarcomas are rare, aggressive soft tissue sarcomas with poor prognosis. In advanced disease, current treatment modalities including chemotherapy and targeted agents, are limited in efficacy and resistance typically ensues. Recent studies have uncovered angiosarcoma subtypes enriched in immune-related signalling and immune cells, including tumours with mast cell infiltration of unclear significance. Therefore, this study aims to investigate the association between mast cell density with clinical features and immuno-oncogenic pathways.

Methods: Immunohistochemical detection of mast cells using anti-tryptase antibody (TPSAB1) was performed on a cohort of 60 angiosarcoma samples. Quantification of mast cell density by H-scores was performed by two independent readers. In addition, NanoString IO360 gene expression data was generated from formalin-fixed paraffin-embedded (FFPE) samples. Correlation of H-scores with NanoString mast cell scores was performed using Spearman's correlation. Survival analyses were conducted using Kaplan Meier curves.

Results: Mast cells were observed in all cases examined and the degree of infiltration ranged from 1% to 90%. H-scores showed a significant moderate correlation with NanoString mast cell

scores (r = 0.53, p < 0.001). Both H-scores and NanoString mast cell scores were significantly associated with head and neck angiosarcomas, non-epithelioid morphology and lower tumour grades (all p < 0.05). A significant but weak correlation with NanoString regulatory T-cell scores was also observed (r = 0.27-0.32, p < 0.05). NanoString mast cell scores positively correlated with signalling pathways relating to antigen presentation, and negatively correlated with apoptosis, DNA damage repair and cell proliferation. Interestingly, for patients with metastatic angiosarcoma, high mast cell density displayed a trend towards reduced overall (HR 2.7; 95% CI 0.96 — 7.61; p =0.0597) and progression-free survival (HR 2.7; 95% CI 0.96 — 7.53; p =0.0586).

Conclusion: We demonstrated the presence of tumour-infiltrating mast cells in angiosarcomas and the involvement of mast cells in specific immuno-oncology pathways. Future research in these pathways may elucidate the roles of mast cells in angiosarcomas and uncover new therapeutic options.

(P 286) EPIGENETIC MECHANISMS AND SIGNATURES OF SARCOMA PROGRESSION AND METASTASIS

Rebecca Dodd, PhD; Qierra Brockman, PhD; Amanda Scherer, MS; Gavin McGivney, BS; Wade Gutierrez, PhD; Jeffrey Rytlewski, MD; Varun Monga, MD, University of Iowa, Iowa City, IA

Objective: Metastasis is a leading cause of death in sarcoma, but the molecular mechanisms driving metastasis are poorly understood. The overall objective of this study is to identify epigenetic mechanisms and signatures of sarcoma metastasis using preclinical platforms and patient datasets. The use of epigenetic modifications to predict tumor progression has not been well-explored in sarcoma due to the complexity of subtype-specific biology and limited number of samples. In our initial study, we determined that loss-of-function mutations in the histone methyltransferase PRC2 can drive MPNST metastasis and remodel the extracellular matrix. We extended these findings to analyses of multi-sarcoma epigenomic patient datasets to demonstrate that epigenetic cross-talk between histone modifications and DNA methyltransferases results in DNA methylation signatures that predict metastasis and tumor progression of multiple sarcoma subtypes.

Methods: To define mechanisms of metastasis in MPNSTs, we used a combination of in vitro metastasis assays, 3D collagen studies and orthotopic mouse models. These approaches were combined with time-lapse microscopy and analysis of patient samples to identify a critical role for PRC2 loss in MPNST metastasis and remodeling of the extracellular matrix. To extend these findings and identify epigenetic signatures of sarcoma metastasis in patient datasets, we used DNA methylation and RNAseq data from patient neurofibromas and MPNSTs with known PRC2 status. Following identification of 8 differentially methylated CpGs in MPNST datasets, we examined these sites in other sarcoma subtypes to determine a DNA methylation signature of metastasis that can be applied to multiple sarcomas.

Results: Loss of PRC2 drives MPNST metastatic phenotypes including collagen-dependent invasion, upregulation of matrix remodeling enzymes, and elevated lung metastases in orthotopic mouse models. Furthermore, clinical sample analysis demonstrates that PRC2 loss correlates with metastatic disease, increased fibrosis, and decreased survival in MPNST

patients. Analysis of epigenomic patient data sets determines that altered histone modifications in MPNST are associated with a global DNA hypermethylation signature. We found over 900 CpGs are hypermethylated in MPNSTs with PRC2 loss. Furthermore, we identified 8 differentially methylated CpGs in Interleukin 17D and Interleukin 17 Receptor (IL17/R) family that correlate with the progression and metastasis of MPNSTs. Changes in IL17D/RD DNA methylation and RNA expression also correlate with progression and metastasis of other sarcoma subtypes including osteosarcoma (OS), rhabdomyosarcoma (RMS), and synovial sarcoma (SS), suggesting these CpGs can predict metastatic outcome in multiple sarcoma subtypes. Analysis of scRNAseq datasets shows that IL17D/RD is expressed in both tumor and stromal cell populations in MPNST, OS and RMS patients.

Conclusion: This study highlights the translational utility of preclinical mouse models to uncover mechanistic drivers of sarcoma biology that inform patient-relevant signatures of sarcoma progression and metastasis. To our knowledge, this is the first analysis of IL17D/RD expression and methylation patterns in sarcoma datasets. These findings have broad implications for disease monitoring and surveillance of multiple sarcoma subtypes. In addition, these data provide a strong rationale for potential targeting of ECM remodeling enzymes and tumor fibrosis to improve outcomes in MPNST patients.

(P 287) ESTABLISHMENT AND GENOMIC VALIDATION OF NOVEL PATIENT-DERIVED XENOGRAFT MODELS FOR DRUG DISCOVERY IN GASTROINTESTINAL STROMAL TUMOR

Lori Rink, PhD; Marya Kozinova, PhD; Martin Belinsky, PhD; Yan Zhou, PhD; Ashot Nazaretian, MS; Kathy Cai, PhD; Sanjay Reddy, MD; Jeffrey M. Farma, MD; Margaret von Mehren, MD, Fox Chase Cancer Center, Philadelphia, PA

Objective: Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors in the gastrointestinal tract. GIST patients often develop clinical resistance to approved tyrosine kinase inhibitors (TKI). The development of effective treatment strategies for refractory GIST requires identification of novel therapeutic targets for investigational agents and well-characterized preclinical models to test these agents. Patient-derived xenograft (PDX) models have potential to better preserve the cellular architecture and molecular complexity of parent tumors. GIST PDX models published thus far have been shown to mirror patient tumors in cell morphology and driver mutation status, but none of these models have been comprehensively profiled at the genome-wide level. Recent studies in other cancers indicate that PDX models may exhibit more nuanced changes at the genomic level upon grafting and passaging in mice. Here, we performed a comprehensive genomic analysis of six KIT-mutant GIST PDX models, all derived from patients previously treated with TKIs.

Methods: Six PDX models were established from recurrent metastatic refractory GIST and maintained in vivo over multiple passages in mice. Along with KIT/PDGFRA genotype determination, histochemical and immunochemical analyses and comparisons of patient to PDX passaged tumors, we performed genome-wide copy number (CN) analysis to define and compare chromosomal aberrations, as well as targeted next-generation sequencing (NGS) profiling with Illumina TruSight Oncology 500 (TSO500), to detect additional mutations in KIT and

Results: Pairwise comparison of copy number variation (CNV) profiles for all samples showed a high degree of correlation between PDX and matched parental tumors. However, four models

demonstrated moderate to strong change in number of CNV events compared to primary tumor. Aberrations in cancer associated genes detected with TruSight Oncology 500 assay showed stability of primary KIT driver mutations, with minor fluctuations in allelic frequencies of acquired mutations across passages, suggesting initial heterogeneity of GIST tumors and potential enrichment of sub-clonal populations through passaging. Preclinical drug testing of these PDX models at various passages is ongoing and will be reported at the meeting.

Conclusion: We have successfully developed six GIST PDX models with diverse treatment histories and genotypes which recapitulate the frequent polyclonal tumors that arise following treatment with multiple lines of TKIs. These tumors represent an unmet clinical need and novel treatment strategies are needed to address this clinical obstacle. Our GIST PDX models preserve a high level of genomic similarity to their corresponding primary tumors in all key molecular features. These fully characterized PDX models have the potential to improve the quality of preclinical testing of novel treatment strategies and to provide further insights into resistance mechanisms to these agents.

(P 288) EVALUATION OF ATR AND WEE1 INHIBITORS COMBINED WITH NANO-LIPOSOMAL IRINOTECAN IN A LARGE COHORT OF RHABDOMYOSARCOMA XENOGRAFTS USING A TWIN MOUSE PRECLINICAL TRIAL DESIGN

Brittney Gordon, BS[1]; Kaley Blankenship, BS[2]; Michael A. Dyer, PhD[1]; Elizabeth Stewart, MD[2], (1) St. Jude Children's Research Hospital, memphis, TN, (2) St. Jude Children's Research Hospital, Memphis, TN

Objective: Rhabdomyosarcoma (RMS) is an aggressive malignancy of childhood which has a poor prognosis when it has metastasized. Although there has been improvement in overall survival for low and intermediate risk patients, there has been no significant change in outcome in the past 25 years for patients with high-risk disease. Previous preclinical studies have shown that RMS orthotopic patient-derived xenografts (O-PDXs) are sensitive to cell cycle checkpoint inhibitors in combination with DNA damaging agents. Here, we compare a WEE1 inhibitor (adavosertib) to an ATR inhibitor (elimusertib) in combination with relevant standard of care agents across 36 O-PDX models (25 embryonal, 10 alveolar, 1 sclerosing).

Methods: In order to encompass the heterogeneity of patient tumors, we used a “twin-mouse” study design. We enrolled two mice for each model from our collection of fully characterized rhabdomyosarcoma O-PDXs (www.stjude.org/CSTN) for every treatment group. Each model was labeled with firefly luciferase for weekly bioluminescent monitoring of tumor burden. The following treatment groups were utilized for testing: placebo, elimusertib + nano-liposomal irinotecan (nal-IRI), adavosertib + nal-IRI + vincristine (VCR), and nal-IRI + VCR. Enrollment was randomized for each of the O-PDXs tested. Four courses (21 days/course) of therapy were given on a clinically relevant schedule using pharmacokinetic guided dosing.

Results: The majority of models (86%) treated with nal-IRI + VCR had some survival advantage compared with placebo. Of the 36 models tested, we found 1 tumor model generated from a patient with refractory disease that failed to respond to any combination (SJRHB013758_X2, ERMS). In contrast, a model generated from the same patient at diagnosis (SJRHB013758_X1) was sensitive to all combinations tested compared to placebo. Similarly, there was a model generated at first relapse (SJRHB013759_X1, ARMS) that responded to

elimusertib + nal-IRI, adavosertib + nal-IRI + VCR and nal-IRI + VCR but subsequent models generated from the same patient at autopsy (SJRHB013759_X14, SJRHB013759_X15) only responded to elimusertib + nal-IRI. There were two models (SJRHB012405_X1, ERMS; SJRHB015720_X1, sclerosing) that responded to both combinations of elimusertib + nal-IRI and adavosertib + nal-IRI + VCR but not to nal-IRI + VCR alone.

Conclusion: The twin mouse experimental design is feasible and represents an attractive strategy for testing diverse models of disease to both identify and prioritize new exploratory combinations. By using two mice to represent each tumor sample, redundancy of each model is provided while still remaining cost effective. By testing a vast collection of RMS O-PDXs in this study, we obtained an understanding of the baseline response of each of the models to standard chemotherapy agents in comparison to the experimental combinations of ATR and Wee1 kinase inhibitors. Furthermore, the identification of resistant models represents an opportunity to better understand the underlying molecular mechanisms of disease resistance and additional studies of these models are warranted.

(P 289) IDENTIFICATION OF EFFECTIVE THERAPEUTIC COMBINATIONS WITH THE NUCLEAR EXPORT PROTEIN INHIBITOR, SELINEXOR, IN PEDIATRIC SOLID TUMORS

Chelsey M. Burke, MD[1]; Diego F Coutinho, PhD[2]; Glorymar Ibanez Sanchez, BS[2]; Andoyo Ndengu, BS[2]; Daoqi You, BS[2]; Armaan Siddiquee, MS[2]; Kristina Guillan, BS[2]; Matthew M. Long, BS[3]; Nestor Rosales, n/a[2]; Paul Calder[2]; Raina Fishkin[2]; Prabhjot S. Mundi, M.D.[4]; Andrea Califano, PhD[5]; Andrew L. Kung, MD, PhD[2]; Filemon S. Dela Cruz, MD[6], (1) Memorial Sloan Kettering Cancer Center, new york, NY, (2) Memorial Sloan Kettering Cancer Center, New York, NY, (3) Memorial Sloan Kettering Cancer Center, Jersey City, NJ, (4) Department of Medicine, Columbia University, New York, NY, (5) Department of Systems Biology, Biomedical Informatics, Biochemistry and Molecular Biophysics, and Medicine, Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY, (6) Memorial Sloan Kettering Cancer Center, Long Island City, NY

Objective: Malignant rhabdoid tumor (MRT) and Wilms tumor (WT) comprise more than 5% of all pediatric cancers. Despite intensive multimodality therapy, outcomes remain dismal for a subset of patients with aggressive or high-risk molecular features. Characteristic of most pediatric cancers, MRT and WT demonstrate relatively low frequencies of somatic mutations compared to adult tumors and generally lack therapeutically targetable genetic alterations. Additionally, conventional monotherapy is often insufficient and more susceptible to the development of resistance in these aggressive tumors. Therefore, our group applies a novel systems biology approach to identify and evaluate non-genetically encoded vulnerabilities that can be targeted in combination in MRT, WT, and other pediatric solid tumors.

Methods: MetaVIPER analysis, an extension of the Virtual Inference of Protein-activity by Enriched Regulon (VIPER) algorithm, was performed to computationally infer protein activity from MRT and WT whole transcriptomic data available in the TARGET database. Expanded metaVIPER analysis demonstrated exportin-1 (XPO1) as having consistently high inferred activity. In vitro cell viability assays utilizing the selective inhibitor of XPO1, selinexor, in combination with other agents targeting proteins identified by metaVIPER were performed in solid tumor cell lines to evaluate the effects on proliferation. Analysis of drug combinations was performed using SynergyFinder 3.0. A malignant rhabdoid tumor cell line was treated sequentially with selinexor and the DNA-damaging agent, doxorubicin, and DNA damage

recovery was analyzed by immunofluorescence using anti-γH2A.X antibody.

Results: MetaVIPER analysis identified consistent high inferred activity of XPO1 in MRT and WT compared to other tumor types. Additional targets, such as topoisomerases (TOP1, TOP2), aurora kinases (AURKA, AURKB), histone deacetylase (HDAC2), and cyclin-dependent kinases (CDK2, CDK4), were also identified as having relatively high inferred activity in MRT and WT (Figure 1). Synergy analyses demonstrated primarily additive responses when selinexor was used in combination with anti-tumor agents in MRT, WT, and other pediatric solid tumor cell lines (Figure 2). The combination of selinexor with palbociclib represents a promising combination in MRT. No combination with selinexor was antagonistic in either the MRT or WT cell lines. The sequential combination of doxorubicin followed by selinexor resulted in delayed recovery from damage caused by the DNA-damaging agent (Figure 3). Selinexor alone did not induce DNA damage. In vivo studies validating sequential and additive/synergistic combinations are ongoing.

Conclusion: XPO1 represents a non-genetically encoded vulnerability in MRT and WT. The sequential combination of a DNA-damaging agent followed by the XPO1 inhibitor, selinexor, results in delayed recovery from DNA damage, suggesting selinexor interferes with the cell's ability to repair DNA damage and drug sequence may be an important consideration for combination therapy with this agent. Furthermore, targeting multiple non-genetically encoded vulnerabilities detected by metaVIPER represents a promising strategy for identifying therapeutic drug combinations. Additional in vivo validation studies are needed to support the development of a pediatric combination clinical trial.

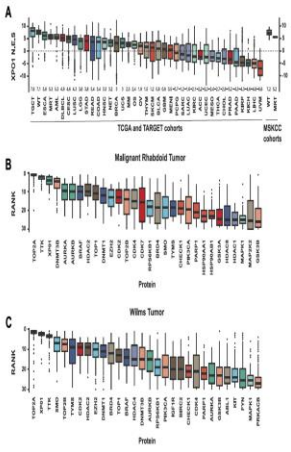


Figure 1. MetaVIPER inference of XPO1 activity in rhabdoid tumors and Wilms' tumor cell lines
(A) Boxplots representing the distribution of metaVIPER inferred XPO1 activity for 33 TCGA tumor cohorts, 3 TARGET pediatric tumor cohorts (MRT, WT, and osteosarcoma), and a neuroendocrine tumor cohort. (B) Boxplots representing metaVIPER protein prediction rank in 68 MRTs and (C) 132 WT from TARGET database.

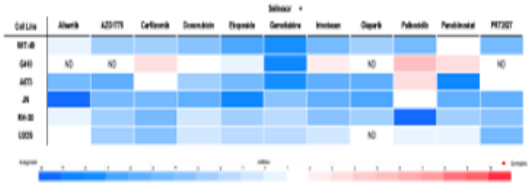


Figure 2. Combination analysis of selinexor plus various anti-tumor agents in pediatric solid tumor cell lines
Six cell lines were screened against several drugs in combination with selinexor. Active drugs were combined with selinexor as non-constant ratio combinations and analyzed using SynergyFinder 3.0. Dose-response curves were fitted using a logistic 4-parameter equation in GraphPad Prism 7. In cases where non-constant ratio combinations were assessed, SynergyFinder 3.0 was used for analysis. Cell lines: WIT49=Wilms tumor; G401=malignant rhabdoid tumor; A673=Ewing sarcoma; JN=desmoplastic small round cell tumor; RH-30=rhabdomyosarcoma; U2OS=osteosarcoma. ND=not done.

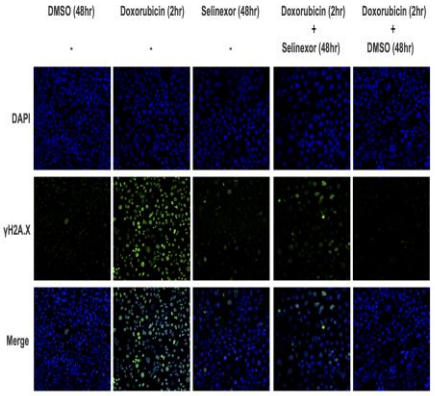


Figure 3. Sequential DNA damage recovery assay in MRT cell line
Immunofluorescent detection of γH2A.X in G401 cells either untreated (DMSO) or treated sequentially with doxorubicin followed by selinexor, or vice versa. DNA damage was detected by staining with γH2A.X. DNA damage due to doxorubicin persisted longer in the presence of selinexor. Selinexor alone did not induce DNA damage.

(P 290) PRE-CLINICAL STUDY OF TUMOR TREATING FIELDS (TTFIELDS) ALONE OR IN CONJUNCTION WITH SYSTEMIC THERAPIES IN SELECTED BONE- AND SOFT-TISSUE SARCOMAS

Herbert Ho Fung Loong, MBBS(HK), PDipMDPath(HK), MRCP(UK), FRCP Edin, FHKCP, FHKAM(Medicine)[1]; Austin ZX Tang, MBChB[2]; Connie Hui, MPhil[1]; Eric Wong, PhD[1]; Gabriel GC Leung, MBChB[1]; Teresa Tse, N/A, MBChB[3]; Vanessa TY Yeung, MBChB[3]; Kingsley Cheung, MBChB[3], (1) The Chinese University of Hong Kong, Hong Kong, N/A, Hong Kong, (2) Hospital Authority, Hong Kong, N/A, Hong Kong, (3) Prince of Wales Hospital, Hong Kong, Hong Kong, N/A, Hong Kong

Objective: Tumor Treating Fields (TTFields) are a novel anti-cancer modality currently approved in glioblastoma and pleural mesothelioma. Clinical trials are on-going in other cancer types. The fields are delivered via the continuous application of low-intensity (1-3 V/cm), intermediate-frequency (100-500 kHz), alternating electric fields to the region of the tumour. When either used alone or in conjunction with cytotoxic chemotherapies or checkpoint inhibitors, TTFields have been well tolerated as reported in large clinical trials and in clinical practice. Although TTFields application in vitro and in vivo was shown to inhibit growth of

various cancerous cell lines, their effect on bone- and soft-tissue sarcoma cell lines have yet been reported. Through in-vitro studies, we investigate potential effects of TTFields in bone- and soft-tissue sarcomas and also investigate the potential for synergism of activities with conventional systemic sarcoma therapies.

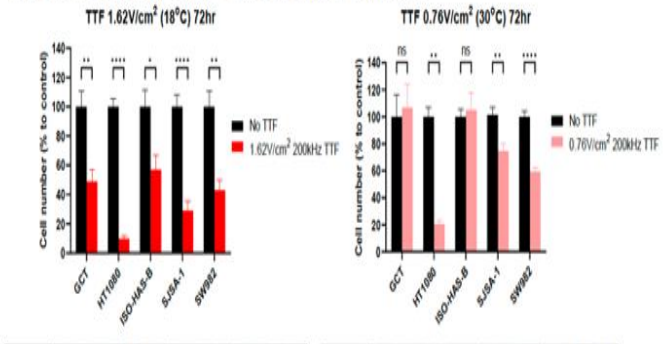
Methods: Five bone- and soft-tissue sarcoma cell lines (GCT, HT1080, ISO-HAS-B, SJSA-1 and SW982) were used in the experiments. According to growth rate of individual cell lines, 20,000 to 30,000 cells were plated on a 22mm diameter round cover glasses and transferred to TTFields ceramic dishes after plating. Cells underwent 72hr 200kHz TTFields treatment. For the control group, the cover glasses were placed in a 6 well plates. All data were obtained in triplicate wells. Cells were collected by trypsinization at end of experiment and counted by either flow cytometry equipment BD AccuriC6 or BioradTC20 cell counter. Two intensities of TTFields (0.76V/cm and 1.62V/cm) were tested. Potential synergism with known active systemic therapeutic agents including doxorubicin and pazopanib were explored.

Results: All cell lines were sensitive to 1.62V/cm TTFields, ranging from 40% to 90% of cells decrease compared with their control cell numbers. Inhibitions were less pronounced in all cell lines but to different extents when treated at lower intensity (0.76V/cm). HT-1080 remained most sensitive even at lower intensity, whereas no effects were observed on GCT and ISO-HAS-B. Moreover, congruent dose-response from nanomolar to micromolar concentrations of doxorubicin in control group (incubated over 72hrs without TTFields) was observed. Preliminary testing for potential synergism between TTFields and doxorubicin showed concentrations of doxorubicin required to achieve similar percentage of inhibitory effect is consistently lower in TTFields-treated vs. TTFields non-treated group. Pazopanib was tested specifically in ISO-HAS-B (angiosarcoma cell line). Potential for synergistic activity was also seen.

Conclusion: TTFields are active in both bone- and soft-tissue sarcomas. Whilst the magnitude of activity differs between different sarcoma subtypes, there is potential for TTFields use in this heterogenous group of diseases as a novel therapeutic option. Moreover, achievement of similar percentage of inhibitory effects despite lower doses of systemic therapies when treated in conjunction with TTFields opens possible strategies for dose optimization of otherwise toxic systemic treatments. This may include the possibility for dose reduction and reduction of dose-intensity thus allowing for prolongation of use of effective treatments which have cumulative non-reversible toxicities, including anthracyclines. Further pre-clinical studies are planned, and clinical window-of-opportunity studies will be necessary to validate this approach.

Funding/Support:
Novocure for support of equipment and consumables to conduct these experiments.

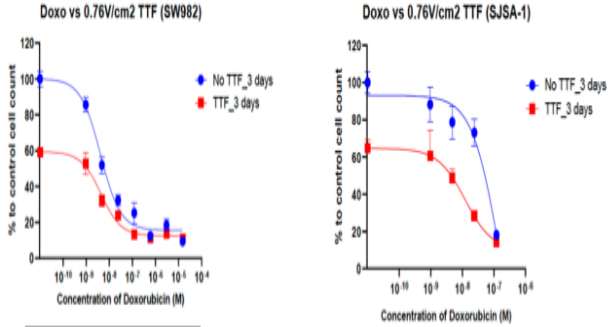
Effect of TTField alone on sarcoma cell lines



	Below threshold?	P value	Mean of No TTF	Mean of TTF_72hr
GCT	Yes	0.001765	100.0	49.01
HT1080	Yes	<0.000001	100.0	9.906
ISO-HAS-B	Yes	0.010774	100.0	56.97
SJSA-1	Yes	0.000007	100.0	29.01
SW982	Yes	0.001477	100.0	42.82

	Below threshold?	P value	Mean of No TTF	Mean of TTF_72hr
GCT	No	0.792676	100.0	106.6
HT1080	Yes	0.003177	100.0	20.51
ISO-HAS-B	No	0.744184	100.0	105.0
SJSA-1	Yes	0.005243	101.4	74.70
SW982	Yes	0.000001	100.0	59.30

Fig 1: Effects of TTFields alone at high- (1.62V/cm) and low- (0.76V/cm) intensities on variety of bone- and soft-tissue sarcoma cell lines



		No TTF_3 days	30GC TTF_3 days
Control	1.00e-011	100.000	59.296
0.96nM	9.60e-010	85.672	52.985
4.8nM	4.80e-009	52.066	32.102
24nM	2.40e-008	32.295	23.890
120nM	1.20e-007	25.160	13.299
600nM	6.00e-007	12.429	11.311
3uM	3.00e-006	10.464	13.935
15uM	1.50e-005	9.447	10.818

		No TTF_3 days	30GC TTF_3 days
Control	1.000e-011	100.000	64.787
0.96nM	9.600e-010	88.218	60.585
4.8nM	4.800e-009	78.742	48.886
24nM	2.400e-008	73.075	28.451
120nM	1.200e-007	18.230	14.623

Fig 2: Dose response curve of SW982 and SJSA-1 cell lines treated with escalating concentration of doxorubicin in doxorubicin and TTFields versus doxorubicin alone groups

(P 291) DEVELOPMENT OF A LIVE TISSUE SARCOMA BIOBANK TO SUPPORT THE CREATION OF PATIENT-DERIVED TRANSLATIONAL MODELS

Merlijn Vekeman[1]; Suzanne Fischer, M.D.[2]; Stefanie Gijsels, MD[3]; Anais Ongenaet, Medical Student[3]; Alexander Ackerman, Medical Student[3]; David Creytens, M.D.[4]; Lore Lapeire, MD, PhD[3]; An Hendrix, dr[3]; Olivier De Wever, PhD[3]; Gwen ML Sys, MD, PhD[2], (1) Ghent University, Zottegem, Oost-Vlaanderen, Belgium, (2) Ghent University Hospital, Ghent, Oost-Vlaanderen, Belgium, (3) Ghent University, Gent, Oost-Vlaanderen, Belgium, (4) Ghent University Hospital, Gent, Oost-Vlaanderen, Belgium

Objective: Sarcomas are malignant mesenchymal neoplasms, of which at least 100 different subtypes have been defined. This sarcoma heterogeneity is a challenge for translational research, as it is not only present between sarcoma subtypes, but also intra-tumoural, meaning that 1 tumour can harbour a multitude of different tumour cells. The combination of this disease's rarity and heterogeneity complicates translational research and the implementation of randomised clinical trials. Our goal is to systematically collect sarcoma tissue samples eligible for the establishment of cell cultures, tissue explants and patient-derived xenografts.

Methods: Patients presented at the multidisciplinary sarcoma team meeting at Ghent University Hospital were offered to participate in tissue collection. Tumour tissue was collected in the operating theatre during biopsy and cut into approximately 1mm3 pieces and frozen to -80°C using a freezing solution of 90% fetal bovine serum and 10% DMSO. Vials were transported to liquid nitrogen for long term storage. The same preservation protocol was used for storage of samples collected during primary tumour resection (only patients without neo-adjuvant treatment) and metastases. Several cell lines, tissue explants and patient-derived xenografts (minimal residual disease models) were created from this biobank following an established protocol developed by our laboratory.

Results: In total, over a period of two years (2020-2022), 155 sarcoma patients were included in the Ghent University Hospital Sarcoma Biobank. The collected samples consist of 52 primary soft tissue sarcomas, 38 primary bone sarcomas, 6 sarcoma metastasis and 57 non-sarcomatous lesions. Of all the sarcomatous lesions included, chondrosarcoma was the most abundant (24 patients), followed by undifferentiated pleomorphic sarcoma (14 patients) and malignant peripheral nerve sheath tumour (10 patients). Cell cultures and PDX models were already created for several patients.The collection is continued further in 2023 and the collection data will be updated again by October 2023.

Conclusion: The Ghent University Hospital Tumour Biobank enables large-scale research on low-incidence, heterogenous sarcomatous tumours. We believe the prospective collection of tumour material may enable further exploration in both the diagnostic and therapeutic approach of patients suffering from sarcoma through cell cultures, tissue explants and patient-derived xenografts.

(P 292) APPLICATION OF HIGH-INTENSITY FOCUSED ULTRASOUND THERMAL ABLATION AND BOILING HISTOTRIPSY WITH CHECKPOINT INHIBITORS IN A MURINE MODEL OF OSTEOSARCOMA: AN IN-DEPTH STUDY

Ahmed Mahran, MD[1]; Pavel Yarmolenko, PhD[2]; Caitlin Tydings, MD[3]; Karun Sharma, MD, PhD[1]; AeRang Kim, MD, PhD[1], (1) Children's National Hospital, Washington, DC, (2) Childrens National, Washington, DC, (3) Food and Drug Administration, Washington, DC

Objective: Despite the significant advances of immune therapy in oncology, its' efficacy has not been appreciated in pediatric solid tumors including osteosarcoma. This may be attributed to an immunosuppressive tumor microenvironment with low mutational burden, diminished immunogenicity, and reduced antigenicity. High intensity focused ultrasound (HIFU) has the potential to improve anti-tumor effects by triggering a localized inflammatory response, enhancing antigen presentation, and promoting T cell activation against tumor antigens. Previous studies demonstrated a survival advantage in treating refractory neuroblastoma using a combination of HIFU boiling histotripsy (BH) and checkpoint inhibitors (CPI), αCTLA-4 and αPD-L1. The objective of this study is to determine if the combination of BH or ablation combined with CPI can offer survival benefit in murine osteosarcoma.

Methods: Osteosarcoma tumors were established following subcutaneous injection of F420 cell line (0.100 x 106 cells) in the hind leg of C57BL/6J mice. Tumor size was monitored until it reached the goal 12 - 15mm across their largest dimension. Mice were randomly assigned to one of 6 experimental groups (n=15 each):1) control, 2) ablation alone, 3) BH alone, 4) CPI alone, 5) ablation + CPI, or 6) BH + CPI. CPI (100μg per dose) were injected intraperitoneally on days 1, 4 and 7 after HIFU or once tumors reached goal size in 'CPI alone' group. Mice were anesthetized and HIFU was performed with a 2000W RF amplifier driving a 1.5 MHz transducer and (BH: 450 mVpp, 13.33ms pulse, 1Hz pulse repetition frequency; ablation: 170mVpp continuous sonication). HIFU was guided by b-mode ultrasound imaging and performed at three adjacent foci (BH: 15 sec/focus; ablation: 5 sec/focus). ELISA for cytokine analysis was performed on serum and flow cytometry was performed on tumors, draining (DLN), contralateral lymph nodes (CLN) and spleen at 24, 48 and 72 h after treatment. Additional mice were used to assess survival (n=10 per group).

Results: No significant benefit was observed between the different treatment groups and control group, with a median survival of 10.5 days and 11 days respectively. Helper T-cells (CD4+ FoxP3-) were upregulated in the DLN following ablation + CPI at 48 and 72 h compared to the control group (40.1% $P < 0.01$, 95% CI [-22.4 to -1.3]). Cytotoxic T-cells (CD8+) were also upregulated in the DLN following ablation + CPI at 48 and 72 h compared to the control group (43.6% $P < 0.001$, 95% CI [-22.1 to -1.81]) (Figure 1). Regulatory T cells (CD4+FoxP3+) were noted in the DLN and CLN following ablation and BH + CPI. BH alone or in combination with CPI led to significantly greater tumor immune cell infiltration (37.5%) compared to ablation alone or in combination with CPI. The CPI alone group had the highest increase in T-cell exhaustion (PD1+ TIM3- and PD1+ TIM3+).

Conclusion: The combination of ablation or HIFU BH with CPI did not improve survival rates compared to control. However, there was significant upregulation in CD4+ and CD8+T cells in the group receiving ablation with CPI. This immune response may highlight the potential of this combination therapy to mobilize the immune system against tumor cells. Furthermore, use of HIFU BH demonstrated improved tumor immune cells infiltration , which may be attributed to its ability to cause mechanical destruction of tumor tissue, potentially exposing a greater array of intact tumor antigens to the immune system. Reduced T cell exhaustion observed in the groups

that received HIFU ablation and BH could be attributed to the mechanical and thermal effects of HIFU to cause tumor necrosis and subsequently stimulate a stronger immune response. Ongoing ELISA analysis of samples to quantify levels of relevant proteins and antibodies may further elucidate immunological alterations triggered by the different treatment modalities and lead to identification of new therapeutic targets for more effective combinatory treatment strategies.

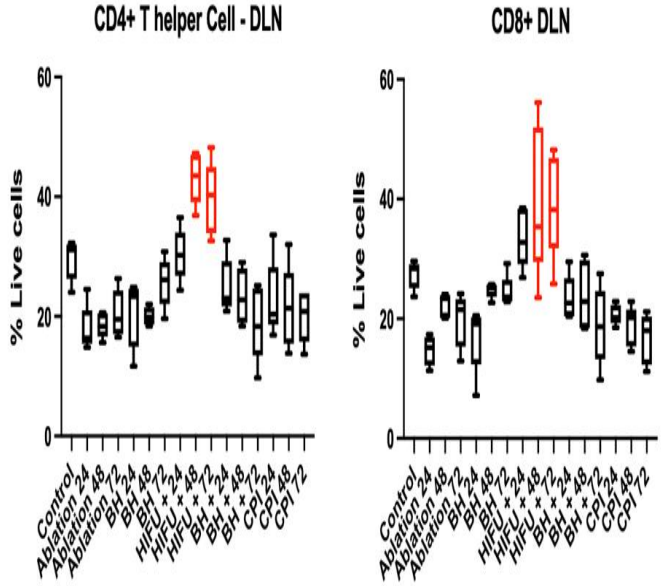


Figure 1. Cellular changes as percent live cells in the DLN evaluated using flow cytometry

(P 293) GENERATION OF A SPONTANEOUS IMMUNOCOMPETENT MOUSE MODEL OF LIPOSARCOMA

Christina V. Angeles, MD[1]; Amanda Shafer, BS[1]; Ahmed M. Elhossiny, BPharm, MS[2]; Jodi Wilkowski, BS[2]; Wei Yan, MD[1]; Scott C. Bresler, MD, PhD[3]; Rashmi Chugh, MD[4]; Arvind Rao, PhD[2]; Andrzej A. Dlugosz, MD[2], (1) Department of Surgery, Rogel Cancer Center, University of Michigan, Ann Arbor, MI, (2) University of Michigan, Ann Arbor, MI, (3) Department of Pathology, University of Michigan, Ann Arbor, MI, (4) University of Michigan Rogel Cancer Center, Ann Arbor, MI

Objective: Liposarcoma (LPS) is one of the most common types of soft tissue sarcoma. The most common subtypes are well-differentiated (WDLPS) and dedifferentiated (DDLPS) liposarcoma which are both characterized in over 90% of cases by genomic amplification of murine double minute 2 (MDM2), a negative regulator of the tumor suppressor p53, while 7% of human WDLPS/DDLPS instead have a TP53 mutation. Patients with metastatic disease have poor overall survival due to the low response rates to systemic chemotherapy. While immunotherapy has shown tremendous efficacy in solid tumors, research focusing on the immune biology of sarcoma has been slower than other tumor types. Genetically engineered mouse models (GEMMs) are needed in an effort to dependably mimic the human stroma and

tumor microenvironment (TME) of liposarcoma. Our lab has set out to develop a spontaneous WDLPS/DDLPS GEMM along with syngeneic cell lines derived from these tumors in order to highly impact the sarcoma community by providing models useful in the pursuit of novel and effective immunotherapeutics for patients.

Methods: A viral construct with an adipocyte-specific promoter sequence driving an eGFP-Myc tag-Cre fusion molecule was used with adenovirus-associated virus (AAV8) vector which elicits low immune response. While PTEN mutations are not found in human LPS, ~80% of DDLPS have phosphorylated AKT. By knocking out Pten, the effect of constitutive phosphatidylinositol-3-kinase (PI3K)-AKT signaling is mimicked. We injected virus into flanks of Ptenf/f;Trp53f/f C57BL/6 mice, resulting in the development of spontaneous tumors. We performed H&E (n=24) and whole exome sequencing (WES) (n=6) on mouse tumors. Bulk RNA sequencing was done on 4 mouse and 9 human DDLPS with matched species-specific normal fat. Gene set enrichment analysis (GSEA) for the Molecular Signatures Database (MSigDb) Hallmark pathways of the differentially expressed genes (DEGs) in both mouse and human DDLPS were compared to respective normal fat. Tumor infiltrating lymphocytes (TILS) were analyzed by flow cytometry and multicolor immunohistochemistry (IHC).

Results: Spontaneous tumors were identified starting at 4 months from AAV8 injection. Histology confirmed all tumors had characteristics of human LPS with 60% DDLS, 10% WDLPS, and 30% mixed WD/DDLPS. Somatic copy number variation (CNV) calling from WES data revealed expected focal deletions of exon 5 in Pten (mean log2ratio = -5.6) and exons 2-5 in Trp53 (mean log2ratio = -5.9), and similar mutational load as published human LPS data. GSEA analysis revealed both mouse and human DDLPS, compared to respective normal fat, showed similar positive and negative pathway enrichment, supporting the significance of this mouse model to human disease (Fig. 1). Flow cytometry revealed 70% CD45+ TILS out of total live cells, of which 21% were CD3+ T cells with equal CD8:CD4 ratio, and 67% were CD11b+ myeloid cells with a small proportion of F4/80+ macrophages. Multicolor IHC confirmed a majority of myeloid cells, however, tumors with the highest number of TILs had areas with increased T cells and greater absolute number of CD8+ T cells compared to those with low TILs. Syngeneic cell lines were generated and consistently produced primary DDLPS tumors when injected orthotopically as well as lung metastasis via tail vein injection.

Conclusion: Primary mouse LPS generated with focally-delivered Cre recombinase and targeted gene deletion mimicked the biological behavior of human LPS and had similar histology, mutational burden, similarly altered pathways evaluated by GSEA, and immune cell subsets in the TME. Syngeneic mouse LPS cell lines can be used for both primary allografts and lung metastasis models in immune competent recipients. This model will be used to determine the key players in immune responses to LPS and preclinical testing of single agent and combination immuno-oncology therapies in order to inform the development of the critically needed effective therapies for liposarcoma patients.

	Mouse		Human	
Pathway	NES	P adj	NES	P adj
Hallmark G2M Checkpoint	1.93	4.2e-07	2.25	4.9e-14
Hallmark E2F Targets	1.88	1.4e06	2.17	2.5e-13
Hallmark Mitotic Spindle	1.58	1.8e-03	1.72	4.1e-05
Hallmark Adipogenesis	-1.95	1.4e-06	-3.63	3.2e-45
Hallmark Fatty Acid Metabolism	-2.3	1.2e-10	-2.97	8.7-21
Hallmark Oxidative Phosphorylation	-2.68	2.2e-19	-3.51	1.0e-40

Fig. 1. Gene set enrichment analysis (GSEA) of the differentially expressed genes (DEGs) in mouse (n=4) and human (n=9) DDLPS compared to species specific normal fat showed similar positive (red arrow) and negative (blue arrow) enrichment of MSigDB Hallmark pathways.

(P 294) DNA-DAMAGE REPAIR DYSREGULATION AND CHEMOTHERAPY RESPONSE IN SARCOMA MODELS

Nils Dieckmann, MD[1]; Dawid Krzeciesa, M.Sc.[2]; Thomas Muehlenberg, PhD[2]; Johanna Falkenhorst, MD[3]; Susanne Grunewald, PhD[4]; Rainer Hamacher, MD[3]; Sabrina Borchert, PhD[5]; Uta Dirksen, MD[6]; Jendrik Hardes, MD[7]; Arne Streitbürger, MD[7]; Hans-Ulrich Schildhaus, MD[5]; Sebastian Bauer, MD[3], (1) University Hospital Essen, West German Cancer Center, Essen, Nordrhein-Westfalen, Germany, (2) The West German Cancer Centre, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (3) Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (4) West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (5) Institute of Pathology, West German Cancer Center, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (6) University Hospital Essen, Pediatrics III, West German Cancer Center, German Cancer Research Center (DKTK) site Essen, National Center for Tumordiseases (NCT) site Essen, ,, Essen, Nordrhein-Westfalen, Germany, (7) University Hospital Essen, Essen, Nordrhein-Westfalen, Germany

Objective: Chemotherapy remains the most important systemic treatment option for most patients with sarcomas harboring complex karyotypes. However, patients do not respond uniformly well to therapy and biomarkers to predict response have not yet been identified. DNA damage repair (DDR) alterations have been shown to be predictive for some chemotherapies in colorectal cancer and brain tumors but its role in sarcomas is unknown. We studied the impact of mismatch repair deficiency (dMMR) and the role of MGMT on chemotherapy response in preclinical sarcoma models.

Methods: We used Cas9-based knockout (KO) to generate isogenic sarcoma cell line models

(originated from myxofibrosarcoma (MFS) and leiomyosarcoma (LMS) cell lines) devoid of MSH2 and MGMT expression. Protein expression was assessed by Western Blot (WB). Microsatellite instability (MSI) was evaluated by Microsatellite replication assay (Promega). MGMT promoter methylation was assessed by methylation-specific PCR. MGMT was overexpressed in a MGMT-negative sarcoma model via lentiviral overexpression. The same MGMT-negative LMS model was continuously treated with temozolomide (TMZ). Cell lines were then characterized for sensitivity to various chemotherapeutic drugs using sulforhodamine B (SRB) cell viability assays.

Results: MSH2 KO caused microsatellite instability (MSI) in one myxofibrosarcoma model. Cell viability assays revealed MSI to confer resistance to nucleoside analoga gemcitabin [GR50: 22 nM vs. > 5 µM – GR50 not reached] and 5-fluorouracil [GR50: 6.6 µM vs. 37.0 µM]. Notably, no shift in sensitivity was observed for other chemotherapeutic durgs (doxorubicin, cisplatin and ifosfamide). MGMT overexpression in a MGMT-negative LMS cell line led to a 5- fold increased TMZ tolerance [GR50: 100µM vs 500µM]. Continuous TMZ treatment of MGMT negative cells resulted in TMZ resistance accompanied by strong endogenous re-expression of MGMT. Notably, MGMT re-expression was not caused by change in MGMT methylation status. MGMT KO in this model reversed TMZ-resistant phenotype. MSH2 KO in MGMT-negative LMS cell line conferred resistance to TMZ.

Conclusion: We here introduce novel, genetically modified in vitro models to study DNA damage repair alterations (dMMR / MSI and MGMT-status) in soft tissue sarcomas. Our data suggest a predictive value of MGMT expression for TMZ/dacarbazine response. Furthermore, MSI appears to be a valid biomarker for the efficacy of nucleoside analoga. We currently investigate the prevalence of these biomarkers in the context of pretreatment as well as correlation with chemotherapeutic response in a retrospective setting.

(P 295) ORTHOTOPIC XENOGRAFTS OF PAIRED SARCOMA PRIMARY TUMOUR, LOCAL RELAPSE AND METASTATIC SITES REVEALS PHENOTYPIC HETEROGENEITY IN A MINIMAL RESIDUAL DISEASE MODEL

Stefanie Gijsels, MD[1]; Suzanne Fischer, M.D.[2]; David Creytens, M.D.[3]; Merlijn Vekeman[4]; Alexander Ackerman, Medical Student[1]; Gwen ML Sys, MD, PhD[2]; Gabriëlle H. Van Ramshorst, MD, PhD[2]; Olivier De Wever, PhD[1], (1) Ghent University, Gent, Oost-Vlaanderen, Belgium, (2) Ghent University Hospital, Ghent, Oost-Vlaanderen, Belgium, (3) Ghent University Hospital, Gent, Oost-Vlaanderen, Belgium, (4) Ghent University, Zottegem, Oost-Vlaanderen, Belgium

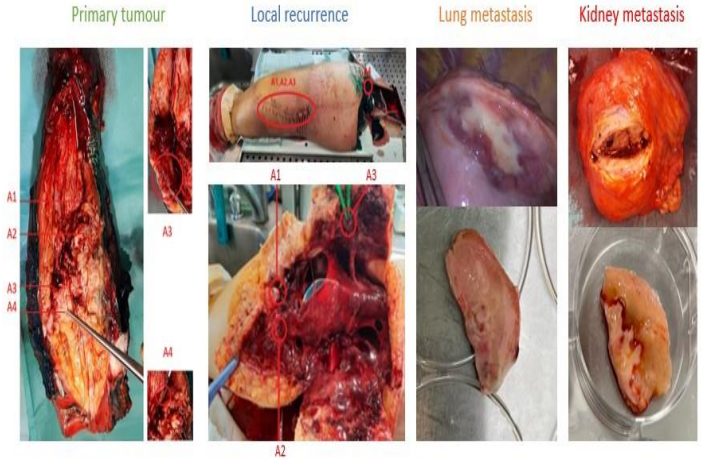
Objective: Soft-tissue sarcoma as a disease is extremely heterogenous, with over 70 distinct histological subtypes. However, there are not only significant differences between the different subtypes, but even within a single patient and a single tumour. We aimed to evaluate the functional differences of macroscopically different regions of a paired primary tumour and its metastatic regions, using a minimal-residual disease mouse model (Fischer, S. et al. - PMID: 37282823).

Methods: A single patient was selected, that suffered from a high-grade malignant peripheral nerve sheath tumour (MPNST). During the disease course, the patient relapsed with a local recurrence followed by a lung metastasis and kidney metastasis. Tissue samples were collected from macroscopically different regions of the primary tumour (4 regions), the local

recurrence (4 regions), the lung metastasis (1 region) and the kidney metastasis (1 region) (figure 1). To create mouse models, we used the minimal residual disease model (MRD-PDX) that was developed by our lab. All 10 tumour regions were implanted in four mice per group to study the primary tumour take ratio and growth rate. Tumour volume measurements were performed using MRI images and 3DSlicer. When the tumours reached a volume of 250 - 500mm^3, the hindlimb containing the primary tumour was surgically resected to achieve minimal residual disease. The animals were then followed until a year after the initial implantation and were sacrificed if humane endpoints were reached earlier. The tumours with the highest take-ratios were reimplanted to create a second generation of mouse models, to test for differences in response to chemotherapy. Once the tumour volume reached 50mm^3, the animals were treated with docetaxel 20mg/kg once a week during 2 weeks and tumour volume was measured as previously described. The animals were sacrificed at two weeks after start of treatment and all organs were collected for pathological examination. All tissue samples were (immuno)histologically assessed by a dedicated sarcoma pathologist.

Results: Of 10 different tumour regions, a take-rate of 100% was seen in two of the primary tumour regions and both metastases. The metastatic potential was highest in the mouse models derived from the patient metastases, with the shortest minimal residual disease status. However, one of the primary tumour regions did show a high metastatic potential, with rapid development of metastases in the lungs and kidneys. This was similar to the patient, indicating the predictive value of the model. Interestingly, histological examination of the mouse metastases of this region revealed a high degree of dedifferentiation and epitheloid-like growth pattern, that looked similar to the patients metastases. Furthermore, the take-rate of the primary tumours was significantly lower than the metastases and there was a clear difference between the primary tumour regions (figure 2). When treated with the same chemotherapy, there was a clear difference in response to treatment between both metastases (figure 3). The experiment is currently still ongoing, so more results will be available in the upcoming weeks.

Conclusion: Heterogeneity within a single tumour and its metastases are maintained under in vivo conditions and the MRD-PDX is a trustworthy instrument to model a patients disease course. We showed that intratumoural heterogeneity affects model development significantly, since models from single biopsies may impact outcome and interpretation. Only one of the primary tumour regions showed a high propensity to metastasise, showing that intratumoural heterogeneity plays a significant role in the disease course of the patient. Furthermore, the differences in treatment response between the two metastases indicate that, even without being treated systemically, a patients metastases can develop a different response profile throughout the disease course.



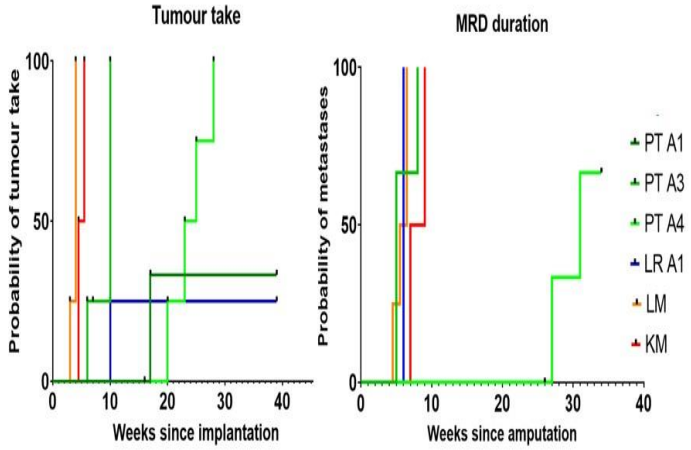
Sampling of the different tumour regions.

Left panel: Primary tumour. A resection of the distal femur and surrounding tissue was performed, with implantation of a megaprosthesis. A1-A4 are the macroscopically different regions that were sampled.

Second panel: Local recurrence. A hip disarticulation was performed. A1-A4 are the macroscopically different regions that were sampled.

Third panel: Lung metastasis. Upper image: a pulmonary wedge resection was performed. Lower image: the sample that was obtained.

Right panel: Kidney metastasis. Upper image: a nephrectomy was performed. Lower image: the sample that was obtained.



Tumour take rate and duration of minimal residual disease (MRD).

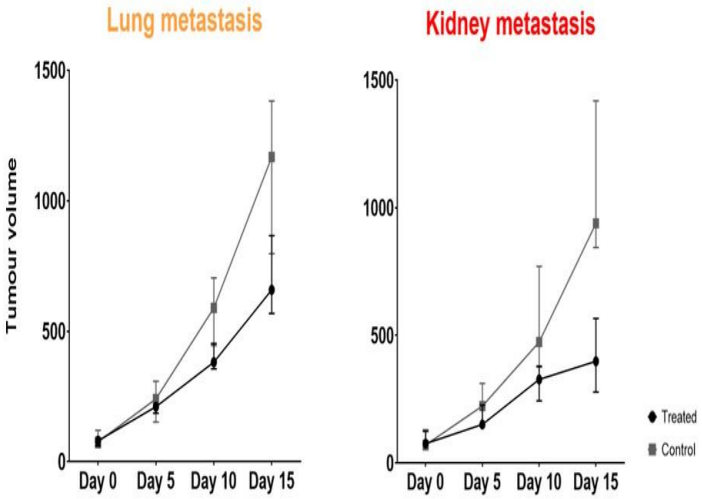
4 animals per group, with a total of 40 mice. The regions that did not grow in the MRD-PDX model are not shown on the graph.

PT = primary tumour; LR = local recurrence; LM = lung metastasis; KM = kidney metastasis. A1 to A4 are the different intratumoural regions as shown in figure 1.

In the KM group, 2 animals were excluded because they were still in follow-up. In the PT A3 group, one animal was excluded because of preliminary death.

Left graph: time until a tumour volume of 250mm³ was reached. At this point, the hind limb containing the primary tumour was amputated.

Right graph: time between hind leg amputation and diagnosis of metastases.



Tumour growth of the second generation mouse models. Treatment (docetaxel 20mg/kg) was intraperitoneally administered once a week during two weeks in the treated group. The control group received vehicle only.
Left panel: tumour growth of the lung metastasis. The growth speed of the treated group was lower than the control group, but the growth curve remained exponential.
Right panel: tumour growth of the kidney metastasis. The growth speed declined significantly and almost reached a plateau phase because of chemotherapy.

(P 296) EX VIVO SARCOMA CELL CULTURES FROM PAIRED PRIMARY, LOCAL RELAPSE AND METASTATIC SITES REVEALS PHENOTYPIC HETEROGENEITY

Stefanie Gijsels, MD[1]; Suzanne Fischer, M.D.[2]; Laurens Boi, n/a[3]; Eline Segers, n/a[3]; Gwen ML Sys, MD, PhD[2]; Gabriëlle H. Van Ramshorst, MD, PhD[2]; Olivier De Wever, PhD[1], (1) Ghent University, Gent, Oost-Vlaanderen, Belgium, (2) Ghent University Hospital, Ghent, Oost-Vlaanderen, Belgium, (3) Ghent University, Ghent, Oost-Vlaanderen, Belgium

Objective: Ex vivo cell cultures from tumour tissue are especially of value for a disease as rare and heterogeneous as sarcoma. Furthermore, on top of the heterogeneity between sarcoma subtypes, there is intratumoural heterogeneity at the level of the primary tumour but also between primary tumour and local relapse and distant metastasis. We aimed to identify biological and functional differences between cell cultures that are derived from a single patient and even a single tumour, to assess the effects of intratumoural heterogeneity.

Methods: A single patient was selected, that suffered from a high-grade malignant peripheral nerve sheath tumour (MPNST). During the disease course, the patient relapsed with a local recurrence and later developed lung and kidney metastases. Tissue samples were collected from macroscopically different regions of the primary tumour (4 regions), the local recurrence (4 regions), the lung (1 region) and the kidney metastasis (1 region) (figure 1). Cell cultures were started from all 10 regions using a dedicated sarcoma cell culture protocol. Morphological analysis was performed using ImageJ. Growth speed was calculated based on cell doublings. A medium throughput drug screening was performed using 35 different drugs in three different concentrations. To validate results of the drug screening, five drugs were selected for GR50 analysis (Hafner M et al, - PMID: 27135972).

Results: Out of 10 tissue samples, 8 cell cultures succeeded and were used for downstream

analyses. Between the cell cultures a significant difference in growth speed and morphotype was observed. A medium throughput screening showed differences in the response profiles of the cell cultures, (figure 2) and the most active compounds were confirmed in a GR50 analysis (figure 3). The cell culture from the lung metastasis clearly showed a different response profile from the cultures of the primary tumour.

Conclusion: From this data, we conclude that the 8 different primary cell cultures show unique phenotypes. The lung metastasis derived cells react differently to chemotherapy, which indicates that a model of the primary tumour is insufficient to determine treatment decisions for a patient with metastases. Furthermore, this research shows that the intratumoural heterogeneity has clinical consequences, since all cell cultures are derived from the same patient but the response to treatment differs significantly. Therefore, it is difficult and incorrect to select a cell culture from one location to represent the complexity of a primary tumour and its metastases.

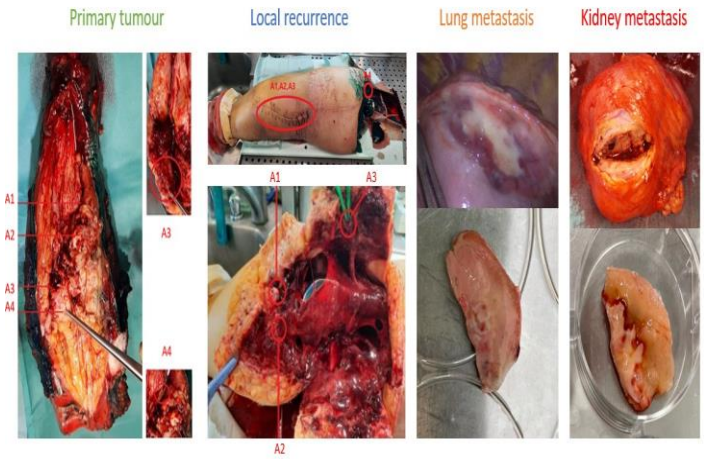


Figure 1. Sampling of the different tumour regions.
Left panel: Primary tumour. A resection of the distal femur and surrounding tissue was performed, with implantation of a megaprosthesis. A1-A4 are the macroscopically different regions that were sampled.
Second panel: Local recurrence. A hip disarticulation was performed. A1-A4 are the macroscopically different regions that were sampled.
Third panel: Lung metastasis. Upper image: a pulmonary wedge resection was performed. Lower image: the sample that was obtained.
Right panel: Kidney metastasis. Upper image: a nephrectomy was performed. Lower image: the sample that was obtained.

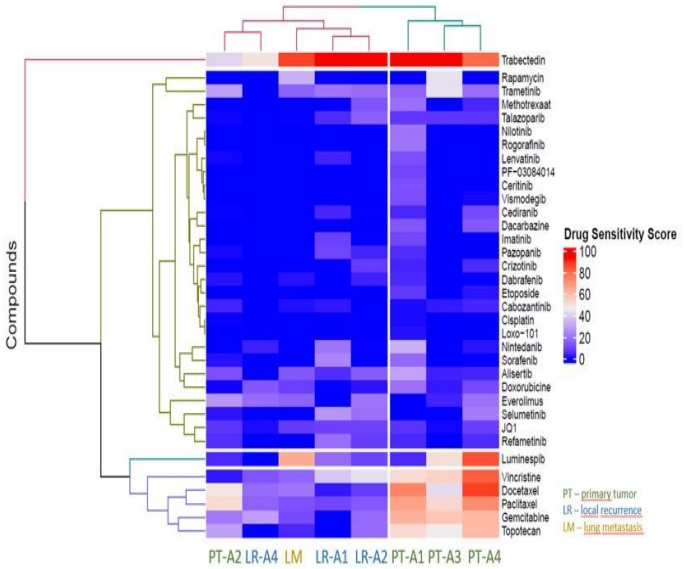


Figure 2. Hierarchical cluster analysis based on Drug Sensitivity Scores (Yadav, B et al - PMID: 24898935). There is a clear difference between the 8 different cell cultures and the lung metastasis shows a different response profile from the other primary cultures.

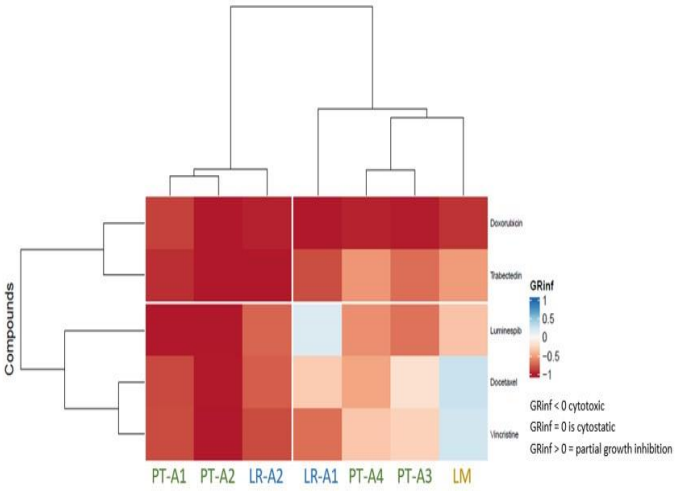


Figure 3. Hierarchical cluster analysis based on GR infinitive values (Hafner M et al, - PMID: 27135972). The best working drugs were validated using a screening protocol in which 11 different concentrations were tested (except for doxorubicin, that was tested in 22 concentrations). PT = primary tumour; LR = local recurrence, LM = lung metastasis. A1 to A4 are the sampled regions as shown in figure 1.

(P 297) UNVEILING THE IMPACT OF INTRATUMORAL MICROBIOTA IN THE TREATMENT EFFICACY OF SOFT TISSUE SARCOMA

Luca Tiraboschi, PhD Student - Biologist[1]; Daniele Braga, Phd[2]; Alessia Melacarne, PhD[1]; Sara Carloni, PhD[3]; Antonino Lo Cascio, PhD[4]; Valentina Ferrari, PhD[5]; Alessandro Mozzarelli, PhD[6]; Giulia Fornasa, phd[7]; Michela Lizier, PhD[8]; Sara Timo, PhD[1]; Giuseppe Martano, PhD, Head of Metabolomics Unit[1]; Giuseppe Penna, Staff Scientist[3]; Alice Laffi, MD[9]; Laura Samà, MD[9]; Laura Ruspi, MD[9]; Salvatore Lorenzo Renne, MD[9]; Ferdinando Carlo Maria Cananzi, MD[9]; Alexia Francesca Bertuzzi, MD[10]; Maria Rescigno, PhD, Research Group Leader[3], (1) Humanitas Clinical and Research Hospital, Pieve Emanuele, Lombardia, Italy, (2) humanitas research hospital, pieve emanuele, Lombardia, Italy, (3) Humanitas University, Pieve Emanuele, Lombardia, Italy, (4) Humanitas Clinical and Research Hospital, Rozzano, Lombardia, Italy, (5) Institute for Research in Biomedicine, Bellinzona, Ticino, Switzerland, (6) NY University, New York, NY, (7) Humanitas Research Hospital, pieve emanuele, Lombardia, Italy, (8) Humanitas Clinical and Research hospital, Pieve Emanuele, Lombardia, Italy, (9) IRCCS Humanitas Research Hospital, Rozzano, Milan, Lombardia, Italy, (10) Humanitas Research Hospital, Rozzano, Lombardia, Italy

Objective: Tumor resident microbiota is an emerging component of the tumor microenvironment with poorly defined biological function. Among all microorganism harbored within tumor tissue, bacteria are currently arising as unexpected factors able to play a role in cancer formation, progression, and response to therapy. This project is aimed to characterize soft tissue sarcoma microbiota and understand if it can impair patients' sensitivity to chemotherapeutic regimens.

Methods: To characterize microbiome composition of soft tissue sarcomas we applied, as an explorative approach, a Fluorescent In-Situ Hybridization targeting the bacterial 16S rRNA and an innovative 16S rRNA gene sequencing method on DNA extracted from sarcoma tissues and adjacent healthy tissues.

To investigate bacterial capability to interfere with anticancer treatments, and particularly with doxorubicin administration, we adopted culturomic approaches to isolate bacteria from fresh sarcoma tissues. We found some bacteria able to metabolize doxorubicin and we confirm their capability to interfere with doxorubicin efficacy in vitro using human and murine sarcoma cell lines, and in vivo with a doxorubicin sensitive murine model of fibrosarcoma (MCA205 injected subcutaneously in C57Bl/6 mice). In addition, products of bacterial biotrasformation of doxorubicin were assessed through mass spectrometry approaches.

Results: We were able to detect bacteria within different histotypes of sarcoma targeting the bacterial 16S rRNA. (Fig.1A) In addition, the 16S rRNA gene sequencing showed that sarcomas arbor a distinct microbiome composition if compared to adjacent healthy tissues. (Fig.1B) Furthermore, comparison of different sarcoma histotypes revealed that microbiome composition seems to be shaped according to histological features. (Fig.1D) We successfully isolated different strains of bacteria from fresh sarcoma tissues, these bacteria were exposed to doxorubicin, and we found that some bacteria have the capacity to completely degrade the drug. (Fig.2A) Functional in vitro experiments confirmed that bacterial exposure can cause, not only the degradation, but also the complete abrogation of drug toxicity. (Fig.2B) In addition, we showed in a doxorubicin sensitive murine model of fibrosarcoma that the presence of doxorubicin-degraders bacteria within the tumor microenvironment promoted the occurrence of chemoresistance.

Conclusion: We revealed that several sarcoma histotypes are colonized by bacteria which constitute the soft tissue sarcoma microbiota. Moreover, we found a direct proof that tumor resident bacteria can degrade doxorubicin impairing the efficacy of chemotherapy. This study offers new insights about the relationship between the intratumoral microbiota and the occurrence of chemoresistance in cancer therapies, suggesting that microbial communities hosted within the tumor should be deeply investigated, and then manipulated, to improve the outcome of therapies.

Table/Chart/image:

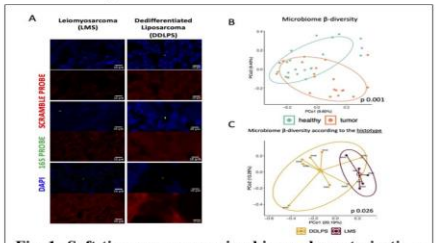


Fig. 1: Soft tissue sarcoma microbiome characterization. A) Representative images of FISH on STS FFPE targeting the bacterial 16S rRNA. B) 16S rRNA gene sequencing data suggested a different microbiome composition between tumors and adjacent healthy tissues (p0.001). C) Sarcoma microbiome distributes in different clusters according to the histotype (DDLPS and LMS, p0.026).

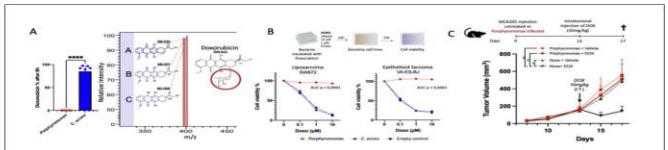


Fig. 2: Intratumoral bacteria inactivate doxorubicin. A) *Porphyromonas* metabolized doxorubicin in 8 hours and main products of degradation identified by Mass Spectrometry. B) Doxorubicin cultured alone or in presence of *C.acnes* showed a dose dependent toxicity which was completely abrogated once cultured in presence of *Porphyromonas*; C) Murine model of fibrosarcoma showed that the presence of *Porphyromonas* within the tumor microenvironment impaired doxorubicin efficacy; (5 mice per group, tumor volume SEM; * $p<0.05$, ** $p<0.01$, 2way ANOVA).

(P 298) EVALUATING THE PHOTOTHERMAL AND CYTOTOXIC PROPERTIES OF INDOCYANINE GREEN IN VITRO FOR INTRAOPERATIVE PHOTOTHERMAL THERAPY IN SARCOMA

Jessica J. Harewood[1]; Corey D. Chan, MBBS, MRCS, MRes, PGCert[2]; Marcus J. Brookes, MBBS, MRes, MRCS[3]; Zoe Bell, MPhys[4]; Kenneth Rankin, MBChB MD FRCS[5], (1) Newcastle University Faculty of Medical Sciences, Romford, England, United Kingdom, (2) Newcastle University, Newcastle upon Tyne, England, United Kingdom, (3) Northern Bone and Soft Tissue Tumour Service, Newcastle upon Tyne, England, United Kingdom, (4) Newcastle University, Newcastle Upon Tyne, England, United Kingdom, (5) Newcastle NHS foundation Trust, Newcastle, England, United Kingdom

Objective: The main objective of this project is to evaluate the heat producing and cytotoxic profile of Indocyanine Green (ICG) when exposed to infrared wavelengths of light. The basis of this investigation is to consider ICG as a photothermal agent for interoperative photothermal therapy (PTT) for sarcoma; performing as adjunct to kill remaining deposits or locoregional micro metastasis of malignant cells after resection. The secondary objectives of this project are: To establish the dark toxicity profile of ICG to confirm the effect of light and demonstrate its

acknowledged safety. To briefly analyse how the parameters of duration of infrared radiation and light intensity impact the efficacy of PTT to help establish an optimal protocol.

Methods: CCK-8 Assay
Chondrosarcoma cell line HT1080 was seeded overnight into 96-well plates at 3.2 x 104 cells per well; following data collected in a seeding density study, ensuring cells were in a consistent growth phase. Plates were treated under a heat lamp bulb from Philips for either: 20 minutes; 10 minutes; 10+10 minutes with a 5-minute intermission; or not treated but left in ambient light. A dimmer switch was used to change intensity of the lamp to halfway or full output. Figure 1. depicts the layout of the experiments. Each plate contained cells exchanged with fresh media after pre-treatment with 0, 0.01. 0.1, 1, 10, 25, 50 or 100uM of ICG for 30 minutes, with 6 replicates at each concentration. For each group of treatment conditions separate plates were analysed at 0h, 24h, 48h and 72h post treatment with a CCK-8 proliferation assay. After an hour incubation with CCK-8 solution absorbance values were measured with an Omega plate reader. For each concentration of ICG absorbance values were compared with the absorbance values for 0uM within the same plate using unpaired t-tests.
Temperature Studies
Solutions of ICG and complete HT1080 media at 0, 0.01. 0.1, 1, 10, 25, 50 or 100uM concentration were placed in a bead bath set to 37ºC for an hour. Using the set-up from figure 1, an infrared thermometer gun was used to take surface temperature measurements of different concentrations of ICG loaded into 6-well plates. Measurements were taken between 0-30 minutes every 5 minutes during full intensity radiation of the heat lamp with each concentration being measured at separate 30-minute intervals. At each time point measurements were recorded three times.

Results: CCK-8 solution reacts with lactate dehydrogenase (LDH) produced by viable cells inducing a colorimetric change proportional to the level of LDH activity in the well increasing the absorbance. CCK-8 absorbance values measured directly after PTT show a slight drop in ICG treated cells compared with 0uM. At 72h post treatment there are more striking differences in absorbance. Figure 2 shows the mean absorbance values collected at 72h for each concentration of ICG. Asterisks denote the significance derived from unpaired t-tests compared against absorbance values for 0uM in the respective plate. * = $P \leq 0.05$. ** = $P \leq 0.01$ *** = $P \leq 0.001$. Noticeably, cells treated at different concentrations of ICG but only left in ambient light do not share the same trend of overt decrease in absorption.
Temperature recordings from two separate temperature studies taken on separate days can be seen in Figures 3&4. Temperatures during PDT increased with time and ICG concentration in both studies. At 0uM there is still a substantial increase in temperature due to infrared radiation from the heat lamp. It is important to note that there was a ~15ºC temperature increase between the weather on the day of the first experiment and the second.

Conclusion: Although this is study is only a preliminary exploration into the effects of PTT with small samples sizes and one cell line; the results are promising and indicate that ICG could be used as an effective photothermal agent for interoperative PTT for sarcoma after further testing.

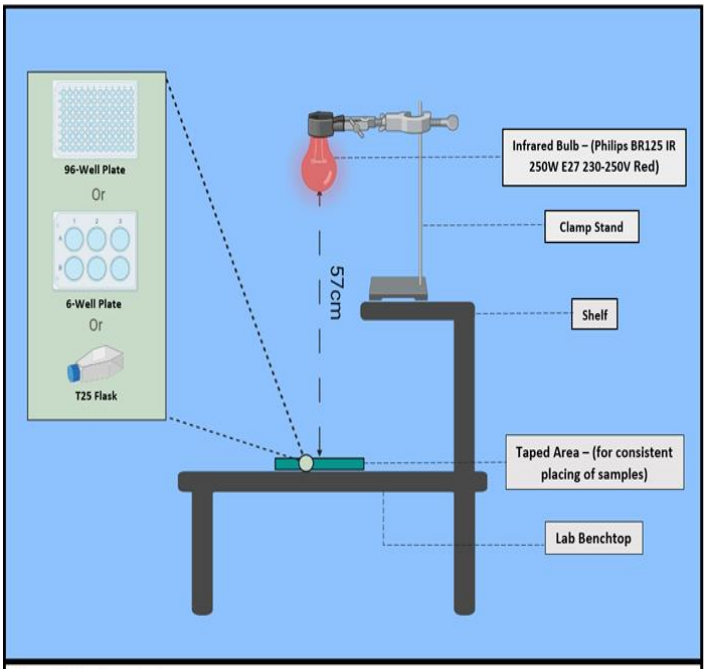
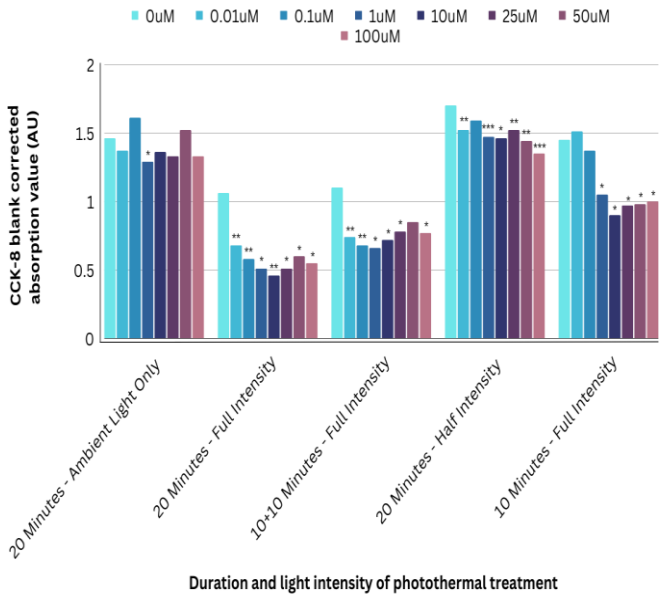
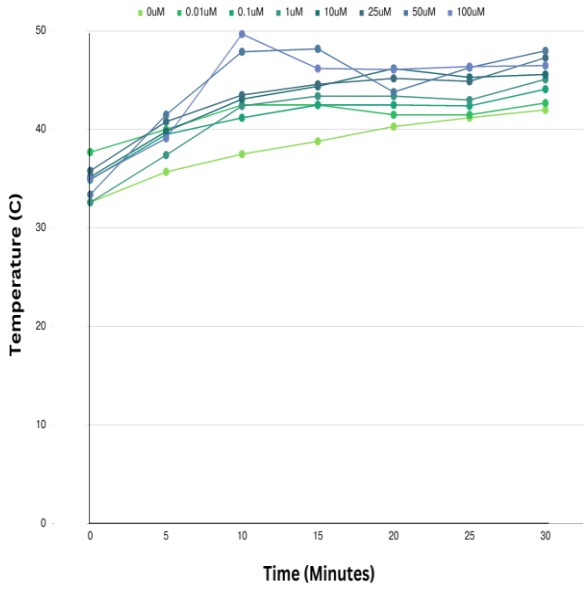


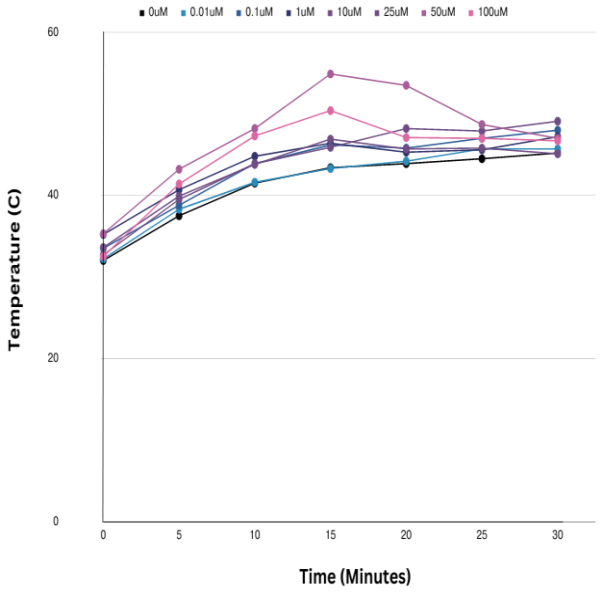
Figure 1: Experiment set-up.



Temperature Experiment 1



Temperature Experiment 2



(P 299) EXPLORING THE IMMUNOLIGHT MECHANISM OF ACTION IN A GENETICALLY ENGINEERED MOUSE MODEL OF SOFT TISSUE SARCOMA

Alexandra Hunter Aitchison, BS[1]; Beatrice Schleupner, BS[2]; Elle M. MacAlpine, MD[3]; Nicole Cantor, BS[4]; Jason A. Somarelli, Ph.D[2]; Brian Brigman, MD, PhD[2]; Julia Visgauss, MD[2]; Michael Monument, MD MSc FRCS(C)[5]; William Eward, MD, DVM[2], (1) Duke University School of Medicine, Durham, NC, (2) Duke University, Durham, NC, (3) Washington University School of Medicine, St. Louis, MO, (4) Campbell University, Mooresville, NC, (5) University of Calgary, Calgary, AB, Canada

Objective: Psoralen is a natural ultraviolet activated compound that has been used for over 70 years to treat skin cancer, psoriasis, and vitiligo. Recently, to expand its use beyond external treatment, a novel therapeutic called X-PACT (X-ray psoralen activated cancer therapy, trade name Immunolight) was created to treat internal tumors. A combination of 8-methoxypsoralen and phosphors that convert low doses of x-ray radiation to ultraviolet (UV) light are injected into solid tumors and subsequently activated by x-ray photons with considerable antineoplastic effect. Notably, canine clinical trials have observed significant abscopal effects of X-PACT therapy on metastatic lesions throughout patients, but the immunomodulatory mechanisms of action are unclear. Here, we present preliminary data investigating the immune response to Immunolight therapy in a murine soft tissue sarcoma (STS) model.

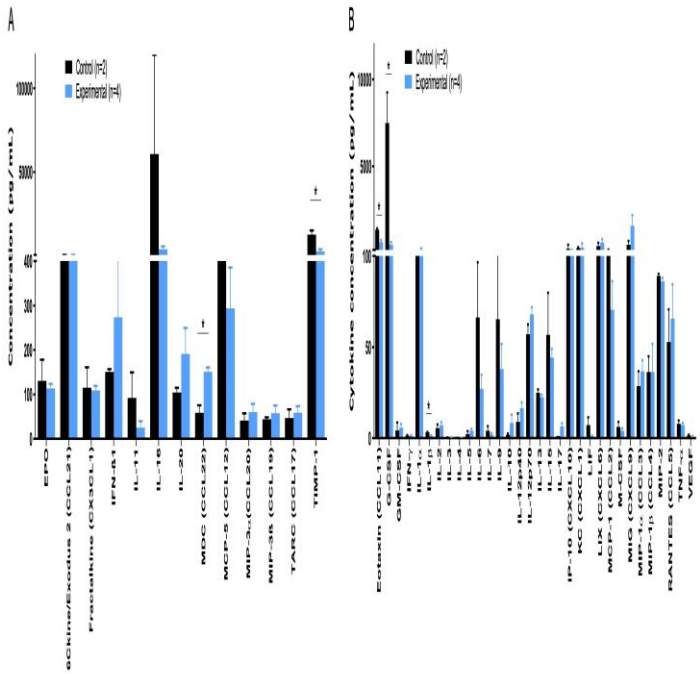
Methods: Six mice of the inducible KrasG12D/+; p53flox/flox (KP) murine STS model with palpable, hindlimb tumors were used in this initial study. Four mice underwent treatment with Immunolight. In this group, psoralen was mixed with energy converters and injected into the tumor. The mice then received low-level radiation to activate the drug. Treatment was performed five times over a 15-day period and the animals were sacrificed within 48 hours of final treatment. The other two mice with induced tumors received no treatment prior to sacrifice. For both groups, a cardiac blood draw was performed at the time of sacrifice to obtain plasma serum for analysis. Luminex xMAP technology was used for multiplexed quantification of 45 mouse cytokines, chemokines and growth factors.

Results: There were five markers that showed significantly different levels between the treated and control groups (Table 1). Results indicated significantly higher ($p = 0.006$) plasma concentrations of myeloid dendritic cells (MDC) following five rounds of treatment with Immunolight therapy. Additionally, the treated mice displayed significantly lower concentrations of granulocyte colony-stimulating factor (G-CSF, $p = 0.003$), eotaxin ($p = 0.016$), tissue inhibitor of matrix metalloproteinase 1 (TIMP1, $p = 0.024$), and interleukin-1β (IL-1β, $p = 0.033$) compared to untreated mice with STS (Figure 1).

Conclusion: In humans, dendritic cells like MDC often drive the immune response to cancer because they prime naïve T-cells with tumor-specific antigens. G-CSF, eotaxin, TIMP1, and IL-1β are upregulated in cancer patients and correlate with disease severity. Together, the lower concentrations of G-CSF, Eotaxin, TIMP1, and IL-1β suggest a softening of cancer severity following Immunolight treatment. Upregulation of MDC in the treated group may indicate the mechanism by which the therapy induces abscopal effects. Further studies and larger sample sizes are necessary to confirm and expand upon these preliminary findings.

Table 1: Multiplex analysis of blood plasma revealed five significantly different markers in the X-PACT treated mice.

Marker	Treated Concentration (pg/mL)	Control Concentration (pg/mL)	P-Value
G-CSF	500.2	7492	0.003123
MDC	151.3	58.79	0.006387
Eotaxin	648.3	1366	0.015875
TIMP-1	2715	12715	0.0237
IL-1β	1.195	3.22	0.032557



(P 300) ANTI-TUMOR ACTIVITY OF PARP7 INHIBITOR RBN-2397 IN SOFT-TISSUE SARCOMAS

Stephanie VERBEKE, PhD[1]; Vanessa CHAIRE, n/a[1]; Aurélien BOURDON, n/a[1]; Jennifer R. Molina, PhD[2]; Antoine Italiano, MD,PhD[1], (1) Institut Bergonié, Bordeaux, Aquitaine, France, (2) Ribon Therapeutics, Boston, MA

Objective: Sarcomas are a heterogeneous group of mesenchymal malignancies that lead to death in about 50% of patients. The prognosis of patients with advanced disease is still poor with a median overall survival of only 18 months. Thus, there is an imperious need to find new therapeutic alternatives in order to improve sarcoma patient's outcome. A recent study investigating the transcriptomic 608 tumors across STS subtypes, established an immune-based classification based on the composition of the tumor microenvironment. The immune-high group characterized by the presence of B cell rich tertiary lymphoid structures (TLS) demonstrated improved survival and a high response rate to PD-1 blockade by pembrolizumab. This work provides novel evidence regarding the presence of immune subtypes in STS patients.
Ribon has developed a first-in-class PARP7 inhibitor RBN-2397 that potently inhibit the growth of cancer cells and restore interferon signaling, effectively releasing the "brake" cancer uses to hide from the immune system and suppress both innate and adaptive immune mechanisms. In several cancer models, RBN-2397 demonstrated durable tumor growth inhibition, potent antiproliferative activity and restoration of interferon signaling. Therefore, PARP7 may represent a new promising therapeutic target that can help to improve the outcome of patients with STS.

Methods: The anti-proliferative and cytotoxic effects of RBN-2397 alone or in combination with cytotoxic agents were first investigated in a panel of 14 STS (soft-tissue sarcoma) cell lines established at Institut Bergonié (Bordeaux, France) using a cell imaging multimode microplate reader, the Cytation 3 technology. Cell cycle phases and apoptosis were then analyzed by flow cytometry and clonogenicity by crystal violet staining. We also assessed the amount of double-strand DNA breaks by STRIDE (SensiTive Recognition of Individual DNA Ends), a highly sensitive technique for the detection of DNA damage in situ in the nucleus and cytosol. Finally, to identify the pathways involved in PARP7 inhibition, we performed a transcriptomic analysis by RNA sequencing in 7 STS cell lines.

Results: We found that all the 14 STS cell lines were sensitive to PARP7 inhibition with RBN-2397 with IC50 range between 60 nM and 12 µM. This sensitivity was characterized by a cytostatic effect with a cell accumulation in G1 phase of cell cycle and a decrease in colony formation without any increase in apoptosis. To assess whether PARP7 inhibition can influence the efficacy of chemotherapy in STS cells, we performed viability assays in combination with either doxorubicin or trabectedin in 10 STS cell lines. Combinations of RBN-2397/doxorubicin or RBN2397/trabectedin were synergistic in 90% and 80% of cell lines respectively, the remaining ones being additive. This synergy is characterized by an increased rate of apoptosis or cell cycle arrest depending on the cell line. To deepen the mechanism of action, we also analyzed the DNA damages in situ by STRIDE technique and found that PARP7 inhibition induces dsDNA breaks in nucleus but also in cytoplasm in some cell lines and that the combination with doxorubicin or trabectedin increased the DNA damages content (Figure 1). Finally, a gene set enrichment analysis (GSEA) after RNA sequencing of 7 STS cell lines revealed an upregulation of several cytokine and TNF signaling pathway as well as an enhanced inflammatory response after treatment with RBN-2397 alone or in combination with chemotherapy.

Conclusion: PARP7 inhibition has anti-tumor activity in soft-tissue sarcomas through cell cycle inhibition and induction of DNA damage. Interestingly, the combination with PARP7 inhibition increases the efficacy of chemotherapy already used in sarcoma management providing a clear rationale for its clinical investigation in this indication.

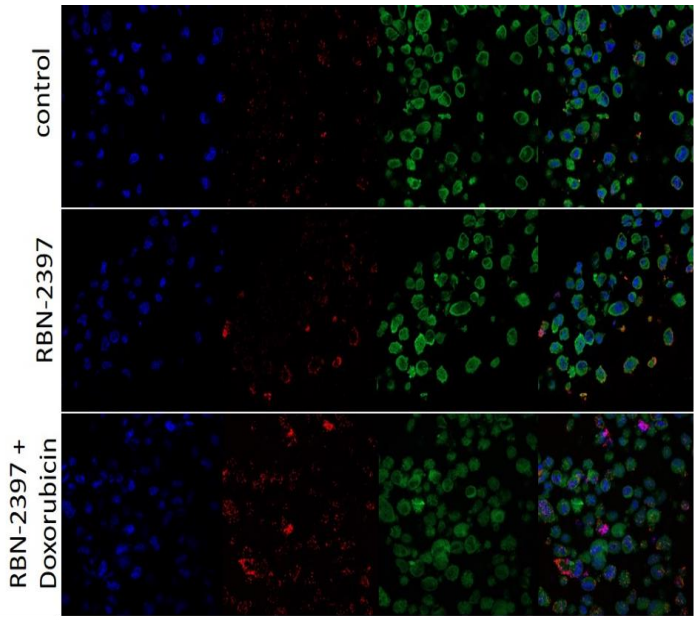


Figure 1: Representative images of STRIDE staining with DAPI (blue), dSTRIDE (red), and GAPDH (green) in JR588 cells.

(P 301) REDUCING THE INVASIVE POTENTIAL OF DEDIFFERENTIATED LIPOSARCOMA BY TARGETING ALTERNATIVE RNA SPLICING MACHINERY

Marina Goryunova, MD[1]; Yiran He, MS[2]; Nipin Sp, PhD[1]; Elizaveta K. Titerina, MD[1]; Sayumi Tahara, PhD[1]; Alessandro La Ferlita, PhD[1]; Michael Sorkin, MD[1]; Alex Kim, MD, PhD[3]; Hua Zhu, PhD[4]; Christopher M. Hadad, PhD[2]; Raphael E. Pollock, MD, PhD[3]; Joal D. Beane, MD[5], (1) The Ohio State University Comprehensive Cancer Center, Columbus, OH, (2) The Ohio State University/Department of Chemistry and Biochemistry, Columbus, OH, (3) The James Cancer Hospital / The Ohio State University, Columbus, OH, (4) The Ohio State University/Division of Cardiac Surgery, Columbus, OH, (5) The James Cancer Hospital / The Ohio State University, Worthington, OH

Objective: Surgical resection is the only curative treatment option for patients with dedifferentiated liposarcoma (ddLPS). However, surgery is often precluded by local invasion of adjacent vital structures or organs. While cytotoxic chemotherapy has been the mainstay of treatment for patients with advanced ddLPS for more than 30 years, response rates are only 25%, and the overall survival at 5 years is 20-30%.
IWS1 is a transcription elongation and RNA processing factor that is activated by phosphorylation of AKT. The mechanism underlying how IWS1 promotes tumor progression is not well understood. Our recent findings demonstrate that IWS1 expression is associated with

worse survival and a shorter time to relapse in patients with ddLPS. Moreso, knocking down IWS1 significantly reduced anchorage-independent growth, migration, and invasion in vitro, as well as reduced the number of tumor metastases in preclinical models of human ddLPS. In this study, we present novel IWS1 inhibitors and provide a rationale for targeting IWS1 pharmacologically as a potential treatment for preventing tumor invasion and metastases in patients with ddLPS.

Methods: To develop an IWS1 inhibitor, we focused on the IWS1/Spt6 interface, as this interaction has been well-characterized and shown to be biologically relevant in literature. Utilizing computer-aided, structure-based virtual screening approaches, we identified a class of compounds that exhibited inhibitory potential. A subset of FDA-approved drugs was evaluated by molecular docking calculations, and compounds with the highest calculated theoretical potency were selected for further analysis.
Lipo863 and Lipo815 cell lines were used as in vitro ddLPS models. To assess whether specific compounds could disrupt IWS1/Spt6 binding, Co-Immunoprecipitation (Co-IP) experiments were conducted. Fraction analysis was performed to examine the effect of a drug candidate on the cellular distribution of IWS1. Finally, to evaluate the impact of IWS1 inhibition on spheroid formation, a matrigel extracellular matrix scaffold was employed.

Results: Through virtual screening, we identified a class of compounds characterized by a specific chemical pattern featuring a tricyclic fused-ring structure (Fig 1). Co-IP of IWS1/Spt6 complex after 48-hour treatment with these drugs showed a decrease of over 50% of complex formation (Fig 2).
Migration of the ddLPS cell line, Lipo863, was reduced by 29% and 18% when treated with Desloratadine or Ketotifen respectively with no effect on cell viability. Moreover, this effect was dose-dependent: 1-10 uM solutions of Ketotifen decreased migration ability by 18-44%, 0.4-4 uM Desloratadine by 34-53% (Fig 3). In addition, the ratio between nuclear and cytosolic fractions of IWS1 increased by 1.1 for 20 uM Ketotifen and by 3.3 for 5 uM Desloratadine compared to DMSO with a ratio of 0.75. In a 3D model, a 14-day treatment with drugs dramatically reduced spheroid formation, confirming the impact on tumor growth and cell proliferation (Fig 4).

Conclusion: From the identified class of potential IWS1 inhibitors, two drugs effectively disrupted the IWS1/Spt6 complex, leading to a compensatory increase in IWS1 expression. Inhibition of IWS1 reduced the migratory potential in vitro and tumor growth in a 3D model. The dose-dependent effect observed for these compounds suggests that there may be an optimal in vivo concentration to reduce cells from invading nearby structures and/or metastasizing. Targeting IWS1 holds potential benefits for preventing recurrence and/or metastases of ddLPS, but in vivo experiments are needed to establish the efficacy of an IWS1 inhibitor. Based on these outcomes, we are currently conducting a follow-up virtual screening to identify additional compounds with greater potency.

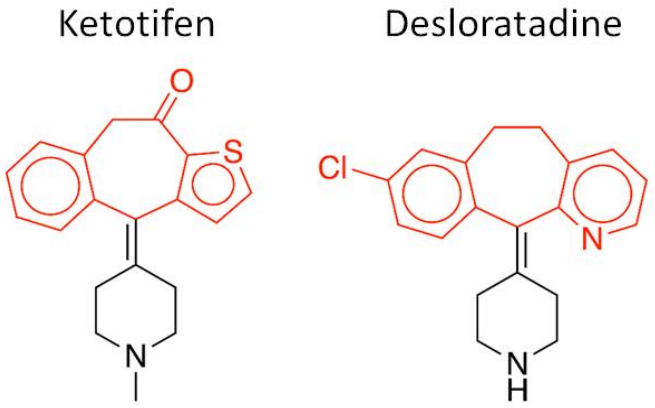


Fig 1. The composition of the tricyclic fused-ring system of IWS1 inhibitors features a central seven-membered ring with two ortho-fused aromatic rings that are five or six membered.

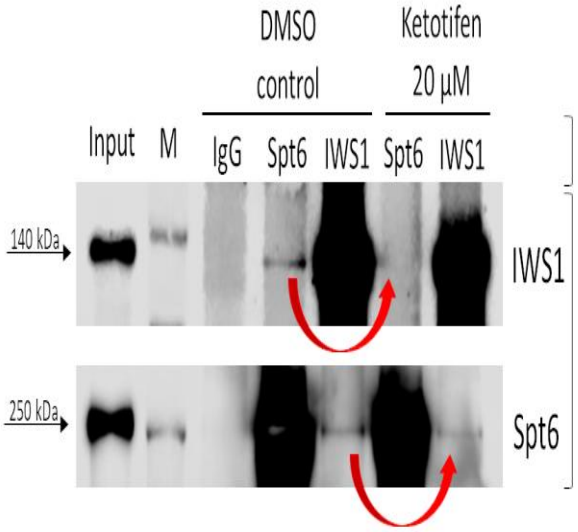


Fig 2. Reduction in the association between Spt6 and IWS1 in Lipo863 cells after treatment with Ketotifen.

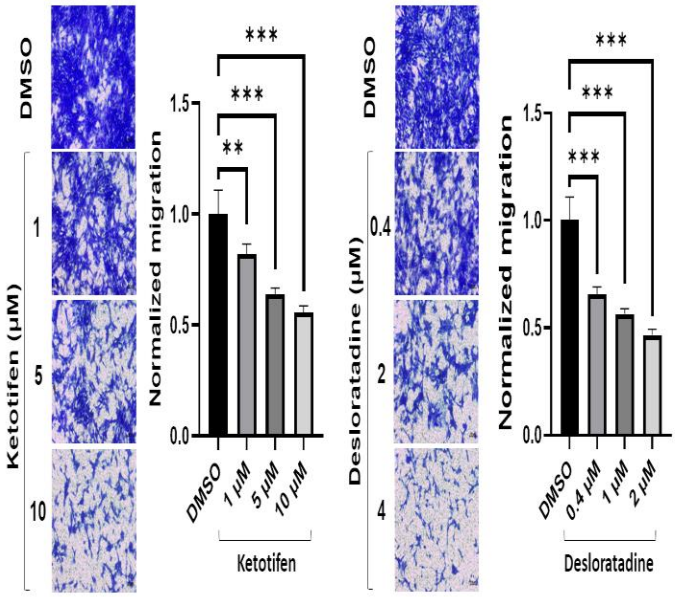


Fig 3. Dose-dependent decrease in migratory ability of Lipo863 cells after 48-hour treatment with Ketotifen and Desloratadine.

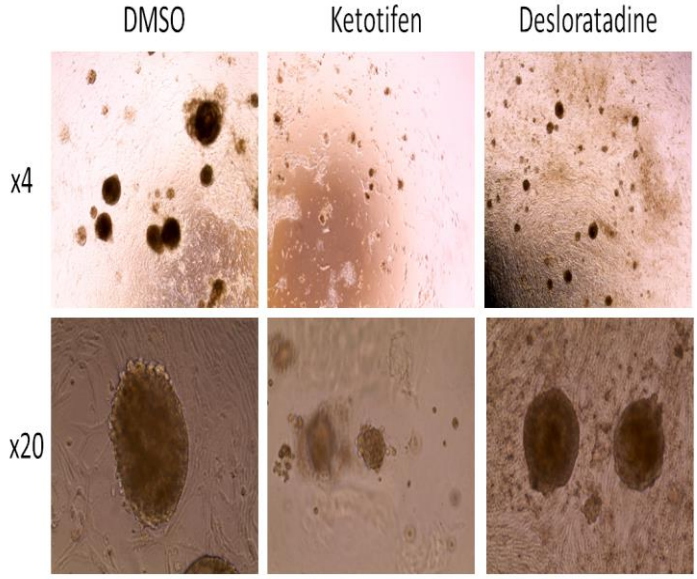


Fig 4. Representative microscopic images of spheroids after 14-day treatment with Ketotifen and Desloratadine.

(P 303) PRECLINICAL EVALUATION OF PARP1 SELECTIVE INHIBITOR AZD5305 IN COMBINATION WITH DNA DAMAGING AGENTS IN EWING SARCOMA ORTHOTOPIC XENOGRAFT MODELS

Elizabeth Stewart, MD[1]; Brittney Gordon, BS[2]; Kaley Blankenship, BS[1]; Burgess Freeman, PhD[1]; Michael A. Dyer, PhD[2]; Sara M. Federico, MD[1], (1) St. Jude Children's Research Hospital, Memphis, TN, (2) St. Jude Children's Research Hospital, memphis, TN

Objective: Ewing sarcoma (EWS) is an aggressive malignancy of childhood with a dismal prognosis in patients with metastatic or recurrent disease. We previously demonstrated that EWS orthotopic patient-derived xenografts (O-PDXs) with the EWS-FLI1 translocation were sensitive to Poly (ADP ribose) polymerase inhibitors (PARPi) combined with DNA damaging agents which was translated to clinical trials in pediatric and adult patients. While responses were seen in patients with EWS, the combination of the PARP1/2 inhibitor talazoparib with irinotecan and/or temozolomide did have a narrow therapeutic window with concern for hematologic toxicity. AZD5305 is a next generation PARPi with 500-fold selectivity for PARP1 over PARP2. Previous studies have shown that PARP2 inhibition may play a role in the hematologic toxicity seen in animal models prompting the study of selective PARP1 inhibitor combinations as an attractive strategy to widen the therapeutic window. We present here the preclinical evaluation comparing the PARP1 selective inhibitor AZD5305 combined with DNA damaging agents nano-liposomal irinotecan (nal-IRI) and temozolomide (TMZ) to similar combinations with talazoparib.

Methods: EWS O-PDXs were created by injecting luciferase labeled EWS cells into the femur of athymic nude mice. Pharmacokinetic studies on tumor-bearing mice were performed to determine matched human AUC-guided dosing and schedule of chemotherapy agents. Preclinical phase I tolerability studies were performed for all drug combinations. EWS O-PDXs were randomized to the following five treatment groups: placebo, AZD5305, AZD5305 + nal-IRI, AZD5305 + nal-IRI + TMZ, and talazoparib + nal-IRI + TMZ. Enrollment was randomized for each of the O-PDXs tested. Four courses of therapy were given on a clinically relevant schedule with each course consisting of 21 days. Bioluminescent imaging was performed weekly to monitor tumor burden and mice were removed from the study and classified as progressive disease at any point when tumor approached 20% of body weight.

Results: Toxicity studies showed that AZD5305 was well tolerated in mice when combined with nal-IRI. Dose reduction was required for tolerability when combined with TMZ; however, this was less when compared to TMZ dose reductions required in talazoparib combinations. Preclinical efficacy studies showed that single agent AZD5305 had no effect on tumor inhibition when compared to placebo. In contrast, AZD5305 + nal-IRI and AZD5305 + nal-IRI + TMZ combinations had significant efficacy in EWS O-PDXs and mice survived all 4 courses of therapy, similar to that of talazoparib + nal-IRI + TMZ. Tumor burden in these treatment groups was not detected by bioluminescence at the end of the study and mice are currently undergoing an observation period to assess for durable response.

Conclusion: DNA damaging agents combined with PARPi have shown effect both preclinically and clinically in EWS; however, the narrow therapeutic window represents a significant challenge in treating patients. The combination of nal-IRI with talazoparib is currently being tested in early phase pediatric trials as one strategy to improve tolerability as nal-IRI combinations were shown in adult studies to have less grade 3/4 dose-limiting toxicities. The development of PARP1 selective agents such as AZD5305 may further mitigate toxicity and allow for dose escalation and potentially improved therapeutic benefit in this patient population.

Further preclinical testing of AZD5305 in additional models is warranted along with combination testing with other novel agents such as ATR inhibitors to help prioritize treatments to move forward in future clinical trials.

(P 304) A COMPARATIVE ANALYSIS OF METABOLIC FLEXIBILITY IN OSTEOSARCOMA CELLS

Anthony Mutsaers, DVM, PhD[1]; Andrew Poon, DVM[2]; Yuan Bie, BSc[2]; Anita Luu, PhD[3]; Alicia Viloria-Petit, PhD[2], (1) Ontario Veterinary College University of Guelph, Guelph, ON, Canada, (2) University of Guelph, Guelph, ON, Canada, (3) University of Guelph, guelph, ON, Canada

Objective: Naturally occurring osteosarcoma (OS) in pet dogs and experimental mouse models provide a translational platform for human OS research. To better understand cellular metabolic changes during OS initiation and progression, we conducted a study to investigate the metabolic signature of canine and murine OS cells, specifically their utilization of glucose versus alternative fuels in central carbon metabolism. We hypothesized that metabolic flexibility post-sarcomagenesis would reveal unique, targetable signatures in the OS landscape.

Methods: We mapped the requirements of primary and metastatic canine, murine and human OS cell lines in comparison with species-specific control mesenchymal stromal cell (MSC) lines. Measurements of the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were obtained with Seahorse respirometry. Cell viability was evaluated after supplementation with glucose, glutamine, pyruvate, or lactate. Canine and murine cells were grown on biomimetic hydrogel cultures with matrix stiffness recapitulative of lung and bone microenvironments, and liquid chromatography with tandem mass spectrometry (LC-MS/MS) of select metabolites was performed.

Results: Canine and murine metastatic OS cells were highly energetic (high OCR/high ECAR) compared to primary OS cell lines and MSCs (low OCR/high ECAR), and preferred glutamine more than glucose or pyruvate, but also consumed the major stereoisomer L-lactate, suggesting a high degree of metabolic flexibility. From LC-MS/MS, the carbon intermediates lactate and malate were heavily co-upregulated in metastatic OS lines, especially in soft lung-like environments (2 kPa), while succinate was comparatively decreased. Citrate release was significantly upregulated in OS cell lines. We later tested the effects of lactate transport inhibition with monocarboxylate transporters MCT1 +/- MCT4 inhibitors AZD3965 and syrosingopine in combination with carboplatin chemotherapy, and found that syrosingopine, but not AZD3965 significantly reduced cell viability.

Conclusion: Collectively, our results suggest that coordinate regulation of lactate metabolism in OS fulfills a metabolic gap in respiration to assist in aerobic cancer survival, and that targeted metabolic inhibitors may synergise with chemotherapy. Furthermore, metastatic OS cells appear to be more energetic and energetically flexible than primary OS cells and mesenchymal cells. Together, these and future investigations into the metabolic landscape of OS may lead to future clinical applications for OS patients.

(P 305) A NEW MOUSE MODEL FOR TESTING SPLICE-SWITCHING THERAPIES IN RHABDOMYOSARCOMA

Dawn Chandler, PhD[1]; Matias Montes, PhD[2]; Safiya Khurshid, PhD[1]; Akila Venkataramany, BS[1]; Timothy P. Cripe, MD, PhD[1]; Peter Houghton, PhD[3]; Enzo Coppola, MD, MD[4], (1) Nationwide Children's Hospital, Columbus, OH, (2) Stanford, Stanford, CA, (3) Greehey Children's Cancer Research Institute, San Antonio, TX, (4) The Ohio State University, Columbus, OH

Objective: Recent advances in high throughput sequencing technologies have uncovered a surprising number of alternatively spliced variants associated with tumorigenesis, implicating regulated splicing in the tumor phenotype. We have shown that the alternatively spliced shorter A variant of the insulin receptor gene (INSR) is highly expressed in pediatric rhabdomyosarcoma (RMS) and can circumvent blockade of the IGF-1R receptor to allow persistent IGF-2 signaling making these cancers resistant to treatment. We have designed antisense oligonucleotides with splice-switching capabilities (splice-switching oligonucleotides, SSOs) to target the INSR pre-mRNA. We hypothesize that these SSOs will restore the normal INSR-B isoform, decrease IGF-2 signaling effects that are facilitated by the shortened INSR-A isoform, and prove to be a promising therapeutic opportunity in mouse models, in the short term, and in human clinical trials and beyond, in the long-term. However, it is necessary to develop animal models that will avail SSO testing in relevant pre-clinical models. Hence, we have turned to the mouse as a powerful tool to generate such a model.

Methods: To generate a new mouse model for RMS that will avail testing of splice-altering therapies in tumors in their native environment (as opposed to cell lines and xenografts), we performed genome editing technologies to induce subtle mutations in intronic splice elements to induce expression of alternatively spliced Insr A in the context of its full-length Insr B counterpart. To test the ability of the mutations to affect the splicing of exon 11, we generated a mouse splice-reporter minigene that includes exon 10, 11 and 12 with intervening introns. We then used site-directed mutagenesis to induce specific mutations that were predicted to disrupt the constitutive splicing pattern of Insr. We generated our mouse mouse mutant line harboring the MBNL G113A/G116A mutation along with a mutation that destroys the protospacer adjacent motif (PAM) to prevent Cas9 cleavage of the template and increase targeting efficiency. Because the G116A and PAM G95A mutants each introduce a unique restriction site, were able screen the resultant mice with PCR followed by restriction digestion. For confirmation, we also sequenced PCR amplicons that span the target mutation site.

Results: We have demonstrated that mouse embryos predominantly express the Insr A isoform, consistent with the splicing INSR ratio seen in human embryonic tissues. Furthermore, we reveal that the muscle tissue expresses the full-length Insr B isoform as early as post-natal day 1, which persists into adulthood. The mouse Insr pre-mRNA splicing is likewise responsive to hypoxia as hypoxic stress induces decreased levels of the mouse Insr B isoform in multiple mouse cell lines. Furthermore, we have established that the Insr splice regulatory elements are conserved in mice and that SSO targeting the CUGBP negative splice regulatory element can restore full-length Insr splicing to yield increased levels of the Insr B isoform. We illustrate that the mutations in the INSR minigenes induced increased levels of the transcript skipping exon 11, with the MBNL G113A/G116A mutant expressing 43% of the smaller transcript, compared to just 4% in the wild-type minigene. Furthermore, Introducing MBNL binding site mutations into the Insr genomic locus results in increased skipping of INSR exon and leads to the expression of stress markers in the muscle.

Conclusion: Our INSR splice-sensitized mouse model can be crossed to existing RMS models to sensitize mice to INSR splicing and RMS. By obtaining an Insr splice-sensitive mouse model we will be able to obtain pre-clinical response data and defining mechanisms of action and biodistribution and targeting of SSOs, we will be well-suited to devise novel, clinically translatable strategies for enhancing cancer therapies.

Radiation Oncology: Updates, Innovations & Controversies

(P 306) USE OF PENCIL BEAM SCANNING PROTON RADIOTHERAPY IN MOBILE SPINE CHORDOMAS

Ivy A. Petersen, MD[1]; Meng Xu-Welliver, MD, PhD[1]; Peter S. Rose, MD[1]; Safia K. Ahmed, MD[2], (1) Mayo Clinic, Rochester, MN, (2) Mayo Clinic Arizona, Phoenix, AZ

Objective: The mobile spine is the rarest site for chordomas and management of these tumors is not uniform involving surgery, radiotherapy or a combination of both. High doses of radiation are required to optimally control these tumors and due to the proximity to sensitive structures proton beam radiation offers an optimal approach. This is an initial review of the use of pencil beam scanning proton therapy (PBPT) in mobile spine chordomas from our institution, presenting tumor response and treatment-associated toxicities.

Methods: Mobile spine chordoma patients treated with PBPT from 3/2016-12/2022 were identified from a prospectively maintained database. A total of 41 primary tumor patients were identified. PBPT was delivered posteriorly for lumbar and thoracic spine tumors typically using 2-3 fields with cervical spine tumors having anterior fields as well. Medical records were retrospectively reviewed for clinical characteristics, surgical details: treatment plan parameters, toxicity, and outcomes under an IRB approved protocol. Descriptive statistics were used to collate patient and treatment characteristics as well as treatment response and toxicity. Recurrences were recorded based on serial imaging.

Results: Location of the chordomas were cervical in 16, thoracic 8, and lumbar 17 patients with a median age of 62 years (range:18-80). All were conventional chordomas except 2 undifferentiated and 1 poorly differentiated. The most common presenting symptom was localized pain (n = 23, 56%) followed by cord compression/cauda equina syndrome (20, 48%) and neuropathic symptoms (radiating pain, numbness, weakness, n= 16, 39 %). Bone and soft tissue disease was present in all cases but one with only soft tissue disease. One patient had local nodal metastases at diagnosis. Mean initial gross tumor volume was 83 cc (range 3-1041). Surgery was performed in 36(88%) patients with radiation done postoperatively in 24, preoperative in 3 and both in 8. Seven patients received radiation as definitive management after biopsy alone. Low-dose photon radiation was used in 5 cases prior to surgery for cord compression. Three patients had P-32 plaque therapy at the time of surgery delivering 8-10 Gy to 1 mm depth along the dura. Surgery was enbloc in 10, marginal resection in 9 and debulking in 17 patients. Most (36, 88%) patients had 2-3 treatment volumes and hypofractionation up to 2.45 Gy/fraction was utilized in 16 patients, one patient was treated b.i.d.

Doses data is outlined in Table 1. Median follow-up after final treatment was 35 mo. (range 0-

76). 11 local recurrences within the radiation volumes were documented at a median of 21 mo.(range 1-53). 3 patients had distant disease: 2 in the lungs and one in lymph nodes. A total of 3 patients have died of disease, all with local recurrences. Treatment-associated toxicities included radiation dermatitis, ongoing pain/stiffness related to treatment, and persistent neuropathies. Chronic pain after treatment was seen in 6 patients.

Conclusion: Mobile spine chordomas are challenging tumors with a variety of treatment approaches. PBPT is an effective component of local therapy often used in combination with surgery. High doses can be achieved utilizing hypofractionation. Optimal integration of radiation with surgery and better standardization of the sequencing needs to be coordinated between surgical and radiation oncology team members.

Table 1

Radiation timing	N	Tumor Location	Median Max tumor dose Gy(Range)	Hypofractionation	Local recurrence
Preoperative	3	1 Thoracic 2 Lumbar	64 (62.5-69.6)	2	1*
Postoperative	25	11 Cervical 5 Thoracic 9 Lumbar	73.5(62.5-77.4)	10	5
Both	7	3 Cervical 2 Thoracic 2 Lumbar	73 (70-80.2)	3	1
Definitive	6	2 Cervical 4 Lumbar	69.6 (69-74)	6	3#

* dedifferentiated chordoma patient
#2/3 Alive with no evidence of disease

(P 307) IS RADIOTHERAPY NECESSARY FOR MANAGEMENT OF LOW GRADE MYXOID LIPOSARCOMA? A SYSTEMATIC REVIEW

Gayathri Vijayakumar, BS[1]; Alexander J. Acuña, MD[2]; Kaitlyn Joyce, BS[1]; Dian Wang, MD, PhD[3]; Alan T. Blank, MD, MS[1], (1) Rush University Medical Center, Chicago, IL, (2) Rush University Medical Center - - Chicago, IL, Chicago, IL, (3) Rush University Medical Center, Chicag, IL

Objective: Myxoid liposarcomas (MLS) are well understood to be radiosensitive soft tissue sarcoma variants. However, there is some institutional variability regarding whether low grade MLS is treated with perioperative radiotherapy. Although there has been increasing interest in neoadjuvant and adjuvant radiation for MLS treatment, the current National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines (CPG) do not support its use to treat low grade MLS. As such, a comprehensive and up-to-date evaluation of the existing evidence is essential to guide clinicians in making well-informed treatment decisions for low grade MLS. This systematic review aims to critically appraise the available literature on the efficacy of radiotherapy in the management of MLS. Furthermore, we will examine how grade and size impact outcomes among MLS patients treated with perioperative radiotherapy.

Methods: The PubMed, EBSCO Host, Cochrane Library, MEDLINE, and Google Scholar online databases were queried to identify studies published between January 1st, 1995 and March 1st, 2023 that evaluated radiotherapy among patients with MLS. After applying our predetermined inclusion and exclusion criteria, a final total of 18 articles were included for our

systematic review. The Methodological Index for Non-Randomized Studies (MINORS) tool was used to assess the quality of included articles. Our analysis included a total of 18 studies reporting on 426 patients (Table 1). The mean (+/- standard deviation (SD)) MINORs score was 11.71 +/- 1.33 for non-comparative studies and 19.25 +/- 2.06 for comparative studies.

Results: Among included studies, there was agreement that pre-operative radiotherapy improved outcomes among patients with MLS (Table 2). Conversely, there was mixed evidence among cohorts receiving isolated post-operative radiotherapy or receiving both pre- and post-operative radiotherapy. A majority of included studies evaluating how tumor size impacted outcomes found that it did not have any effect on local recurrence, disease specific survival, or distance metastasis rates. A large majority of articles commenting on tumor grade indicated that a higher grade on French Federation of Cancer Centers (FNCLCC) grading system and a round cell component >5% were associated with a higher risk of local recurrence and metastases and a lower rate of overall and disease specific survival.

Conclusion: Our systematic review aims to help guide providers regarding the efficacy of pre-operative radiotherapy prior to surgical resection of MLS. Similarly, our analysis found that higher tumor grade was associated with worse outcomes while size did not appear to have an effect. It is important to note that there were limited numbers of high-quality studies in our review. Similarly, although included low grade MLS patients were treated with radiotherapy, there is a paucity of information regarding how radiotherapy impacts outcomes in isolated low-grade MLS cohorts. Given the trend in treating MLS, including low grade MLS, with radiotherapy, we have highlighted a gap in the existing evidence to determine the necessity of radiotherapy in low grade MLS patients. Future studies should evaluate the utility of radiotherapy in this cohort as well as in patients with low grade MLS less than ten centimeters.

Table 1. Overview of Article Included in Final Review

Author	Year Published	Study Design	MLS characteristics	Number of Patients who Received Radiotherapy
Nishida et. al	2010	retrospective review	Pure MLS defined as <5% RCC	Preop: None Postop: 9
Haniball et. al	2011	retrospective review	Pure MLS (defined as no RCC, <5% round cell component and >5% RCC	Preop: None Postop: 107
Houdek et. al	2023	retrospective review	Pure MLS (defined as no RCC), <5% round cell component and >5% RCC	Preop: 62 Intraop: 21 Postop: 7
Büyükceran et. al	2022	retrospective review	Pure MLS (defined as no RCC), <5% round cell component and >5% RCC	Preop: 14 Postop: 29
Perera et. al	2022	prospective study	<5% round cell component and >5% RCC	Preop: 198 Postop: None
Lansu et. al	2021	nonrandomized controlled trial	<5% round cell component and >5% RCC	Preop: 77 Postop: None
Koseła-Paterczyk et. al	2020	nonrandomized clinical trial	<5% round cell component and >5% RCC	Preop: 27 Postop: None
Tsuchie et. al	2020	retrospective study	*No comment on RCC	Preop: None Postop: None
Salduz et. al	2017	retrospective study	Pure MLS (defined as no RCC), <5% round cell component and >5% RCC	Preop: 23 Postop: None
Koseła-Paterczyk et. al	2016	prospective study	<5% round cell component and >5% RCC	Preop: 32 Postop: None
De Vreeze et. al	2008	retrospective study	pure MLS (no RCC)	Preop: 8 Postop: None
Baxter et. al	2014	retrospective study	Pure MLS= <5% round cell component	Preop: 38 Postop: 20
Moreau et. al	2012	retrospective study	<5% round cell component and >5% RCC	Preop: 170 Postop: 118 Preop and Postop: 21
Chung et. al	2009	retrospective study	<5% round cell component and >5% RCC	Preop: 41 Postop: 38 Preop and Postop: 9
Guadagnolo et. al	2008	retrospective study	*No comment on RCC	Preop: 68 Postop: 59
Smith et. al**	1996	retrospective study	Pure MLS (defined as no RCC) , <5% round cell component and >5% RCC	Preop or Intraop or Postop: 11
Kilpatrick et. al	1996	retrospective study	Pure MLS (defined as no RCC) , <5% round cell component and >5% RCC	Preop or Intraop or Postop: 38
ten Heuvel et. al**	2006	retrospective study	Pure MLS (defined as no RCC) , <5% round cell component and >5% RCC	Preop and Postop: 2 Postop: 22

Table 3. Overview of Article Included in Final Review

Study	Key Findings
Nishida et. al	Local recurrence rates were higher in patients receiving radiotherapy (22% vs. 11%). However, 5-year survival was not significantly difference between those with and without radiotherapy (83% vs. 91%; p=0.85). Radiotherapy and tumor size did not have an impact on either overall or disease-free survival.
Haniball et. al	There was no effect of radiotherapy (p=0.80), size over 5 cm (p=0.476), or location on the risk of local recurrence. A round cell component over 5% was significantly associated with risk of local recurrence (p=0.03). Patient's receiving radiotherapy had a higher rate of 5-year metastases compared to patients without post-operative radiotherapy (36% vs. 28%). However, this was not significant (HR: 0.725, 95% CI: 0.38-1.38; p=0.329). There was no effect of size, location, or site on the risk of metastases. A round cell component of 0-5% (HR: 4.166, 95% CI: 0.136-12.78; p=0.0126) and of >5% (HR: 8.899, 95% CI: 3.35-23.64; p<0.0001) were associated with a significantly higher risk of metastases at 5-years.
Houdek et. al	Radiosensitive tumors were shown to have a significantly lower risk of disease specific death (HR: 0.22, 95% CI: 0.06-0.77; p<0.01). This was similarly seen when evaluating local and distance recurrence with radiosensitive tumors (HR: 0.21, 955 CI: 0.08-0.55; p<0.001) showing a lower risk of recurrence compared to non-radiosensitive tumors. Tumor size over 10 cm (p=0.26) and a round cell component (0.99) did not significantly impact disease specific survival.
Büyükceran et. al	Patients in the neoadjuvant RT group had no significant difference in overall survival (p=0.349) or disease-free survival (p=0.691) compared to the adjuvant RT group. Tumor size of ≥10 cm (p=0.045), round cell component >5% (p=0.041), and high-grade tumor (p=0.022) were significantly associated with lower survival (p=0.045). Tumor size ≥10 cm had a trend for significance for decreased disease-free survival (p=0.076).
Perera et. al	A round cell component >5% (p=0.002), pre-treatment estimated tumor volume over median size of 240 cm (p=0.003) and decrease in estimated tumor volume by less than 60 % (p=0.004) were all significantly associated with metastasis. A round cell component >5% (p=0.009) and decrease in estimated tumor volume by less than 60 % (p=0.041) were significantly associated with decreased overall survival.
Lansu et. al	No local relapses occurred at median 25 months following surgery.
Koseła-Paterczyk et. al	No patients with low or high grade developed local recurrence following RT. No low-grade patients went on to develop metastatic disease at maximum follow-up. Higher tumor grade (p=0.00074) and increasing round cell component percentage (p=0.009) were significantly associated with decreased disease-free survival.
Tsuchie et. al	AJCC stage (p=0.0009), tumor size(p=0.035) and surgical treatment (p=0.019) for metastasis were significantly associated with reduced overall survival.
Salduz et. al	Round cell component demonstrated a trend towards significance with development of metastasis (p=0.054). 5 year metastasis free survival in patients with round cell components was 27% compared to 79% in patients without round cell component. Tumor size greater than 15 cm (p=0.006) was significantly associated with development of metastasis. Tumor size greater than 15 cm (p=0.038) and metastasis (p=0.001) were significantly associated with worse overall survival. Round cell component was not significantly associated with overall survival although overall survival was 53% in patients with round cell component compared to 85% in patients without round cell component.
Koseła-Paterczyk et. al	There were no statistically significant differences in terms of local recurrence free survival for round cell component, tumor grade, or tumor size.
De Vreeze et. al	No local recurrences occurred at 20 months median follow up.
Baxter et. al	Increasing age (p=0.04) and increasing tumor size (p<0.01) were the only significant predictors of recurrence. Radiation (p=0.37) was not a significant predictor of recurrence free survival.
Moreau et. al	Radiotherapy (p<0.001) and the margin status (p<0.001) were independent predictors of local recurrence. Radiotherapy prevented local relapse (p < 0.001) and also reduced by sevenfold the 5-year local recurrence rate in patients with positive margins (P < 0.05). No significant relationship was found between tumor size greater than 10 cm and local recurrence. Round cell component >5% (p=0.003), higher tumor grade (p=0.028), tumor size over 10 cm (p=0.01) were independent prognostic factors for development of metastasis.
Chung et. al	Only two of the 88 MLS patients developed local recurrence at median 61 months. Both patients had positive resection margins. One of the patients received preoperative and postoperative radiation and the other patient received radiation only postoperatively. Overall survival at 5 years was significantly higher in patients with MLS compared to other soft tissue sarcomas (93.9% vs 76.4%; p<0.01).
Guadagnolo et. al	Tumor size, margin status, and tumor site were not significantly associated with distant metastasis.
Smith et. al	In seven patients with pure MLS, there were three (42.8%) local recurrences. One patient had an 11 cm MLS and received RT and surgery. The other patient had an 8 cm MLS and only received surgery. The third patient had a 5 cm MLS and only received surgery. Two (29%) of the seven patients with pure MLS developed metastasis and both patients had surgery and RT for the primary tumor.
Kilpatrick et. al*	Tumor location and poor surgical margin were significantly associated with local recurrence. No statistical difference was seen in local recurrence between patients treated with marginal resection and radiation compared to wide resection alone. Primary tumor 10+ cm, older age (45+), and round cell component over 25% were significantly associated with development of metastasis. Age over 45 and round cell component over 25% were significantly associated with decreased overall survival.
ten Heuvel et. al	Tumor location was significantly associated with local recurrence (p=0.003). Round cell component >5% was significantly associated with worse disease specific survival. Tumor size greater than 5 cm was not significantly associated with disease specific survival. In patients with R1 or marginal R0 resection that were eligible for radiation, 57% received radiation and there was a statistically significant increase in local recurrences in patients that did not receive radiation (p=0.01).

*LR in this cohort was only measured in 55 pt

(P 308) OPTIMAL BIOLOGIC EFFECTIVE DOSE OF STEREOTACTIC BODY RADIOTHERAPY FOR METASTATIC AND RECURRENT SARCOMA

Erin S. Murphy, MD[1]; Jenna Kocsis, MD[1]; Eashwar Somasundaram Somasundaram, MD[2]; smile Timothy, MD[3]; Jacob G. Scott, MD PhD[4]; Lilyana Angelov, MD[1]; Matteo Trucco, MD[1]; Peter M. Anderson, MD, PhD[1]; Stacey Zahler, N/A, MD[1]; Stephanie THomas, MD[1]; Peng Qi, PhD[1]; Anthony Magnelli, PhD[1]; Shauna R. Campbell, DO[5], (1) Cleveland Clinic, Cleveland, OH, (2) Case Western Reserve University, Cleveland, OH, (3) St. Francis Medial Center, Peoria, IL, (4) Cleveland Clinic, Gates mills, OH, (5) Cleveland Clinic Foundation, Cleveland, OH

Objective: Patients with metastatic sarcoma can be cured if aggressive local tools are used in addition to systemic therapy. Stereotactic body radiation therapy (SBRT) affords the potential to overcome the relative radioresistance of sarcomas through delivery of a focused higher biological effective radiation dose (BED) as an alternative to invasive surgery or conventional radiotherapy. Our current SBRT regimen was based on a small institutional series and dose not vary by sarcoma histology. We sought to understand the local control of sarcoma SBRT as it relates to histology and to identify an optimal SBRT regimen.

Methods: From our IRB-approved single-institution registry, all patients treated with SBRT for metastatic sarcoma between 2014 and 2020 were identified. A receiver operating characteristic (ROC) curve was generated to determine optimal BED. Local control was compared by SBRT dose using the BED cut point and evaluated by histology. The Kaplan-Meier method was used to compare local control rates first by radiosensitivity and then by dose treatment groups. A stratified Kaplan-Meier analysis then analyzed the interaction of radiosensitivity and dose on local control. Log-rank statistics were used to compare the groups.

Results: Forty-two patients with a total of 138 lesions met inclusion criteria. Median SBRT prescription was 116.70 Gy BED (range 66.70 - 419.30). Desmoplastic small round cell tumor, Ewing sarcoma, rhabdomyosarcoma, and small round blue cell sarcomas were classified as radiosensitive (n= 63), and all other histologies were classified as radioresistant (n=75). Local control for all lesions was 66.7% (95% CI, 56.6-78.5) at 1 year and 50.2% (95% CI, 38.2-66.1) at 2 years. Stratifying by histology, 1- and 2-year local control rates were 65.3% and 55.0%, respectively, for radiosensitive, and 68.6% and 44.5%, respectively, for radioresistant histologies (p=0.49). The ROC cut point for BED was 95 Gy. Local control rates at 1- and 2-years were 75% and 61.6%, respectively, for lesions receiving >95 Gy BED, and 46.2% and 0%, respectively, for lesions receiving < 95 Gy BED (p=0.01). On subgroup analysis, local control by BED >95 Gy was significant for radiosensitive histologies (p=0.013), and trended toward significance for radioresistant histologies (p=0.25). A separate analysis was performed specifically for osteosarcoma patients (23) and lesions (66). The 6- and 12-month local control rates were 89% (95% CI 0.8-0.99) and 78% (CI 66%-92%) respectively. A dose cutoff for improved local control was found at a BED of 120 Gy. Local control at one year was 89% (CI 77%-100%) for lesions that received ≥120 Gy BED versus 61% (CI 40%-91%) for those that received < 120 Gy BED (p=0.034). No acute grade ≥3 toxicity was observed for any of the patients.

Conclusion: Our large sarcoma SBRT series demonstrates that local control for recurrent and metastatic disease is possible with minimal toxicity. Local control was significantly improved

with a biologically effective dose of at least 95 Gy for radiosensitive histologies and 120 Gy for osteosarcoma. These SBRT regimens should be further investigated on prospective trials

(P 309) CLINICAL PATHOLOGICAL CHARACTERISTICS, PATTERN OF CARE AND SURVIVAL OF NON-METASTATIC BREAST ANGIOSARCOMA

Daniela Greto, MD[1]; Mauro Loi, MD[1]; Icro Meattini, MD[2]; Ilaria Bonaparte, MD[2]; Annarita Palomba, MD[3]; Domenico Andrea A. Campanacci, Full Professor[4]; Lorenzo Livi, Full Professor[2], (1) Radiation Oncology Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy., Florence, Toscana, Italy, (2) Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy, Florence, Toscana, Italy, (3) Unit of Histopathology and Molecular Diagnostics, Careggi University Hospital, Florence, Italy., Florence, Toscana, Italy, (4) Department of Orthopaedic Oncology and Reconstructive Surgery, Azienda Ospedaliero Universitaria Careggi, Firenze, Italy, Florence, Toscana, Italy

Objective: Breast Angiosarcoma (BAS) is a rare soft tissue sarcoma subtype that may arise de novo or in previously irradiated patients. Due to its scarce incidence, prognostic factors and optimal pattern of care are ill-defined. The aim of this study is to investigate the clinicopathological features, treatment modalities and outcome of BAS

Methods: Clinical and treatment-related data from consecutive non-metastatic patients treated at our Institution from June 2006 to December 2020 were retrospectively reviewed. Local control (LC), Distant Metastasis-Free Survival (DMFS), Overall Survival (OS) were calculated using the Kaplan-Meier method and compared with log-rank test.

Results: Twenty-two patients were included in the analysis. Median age was 60 (17-86) years. In 15 patients, AS arose in a previously irradiated field following adjuvant radiotherapy of prior breast adenocarcinoma. No information on pre-existing edema were available. All patients underwent surgery consisting of wide excision and mastectomy in 6 and 16 cases respectively.Grade 3 FNCLCC disease was identified in 7 patients. Postoperative radiotherapy and chemotherapy were required in respectively 5 (median dose 60 Gy, range 25-66 Gy) and 3 patients. At the time of our analysis after a median follow-up of 36 months disease recurrence occurred as local, distant, and concurrent distant and local failure in 3, 6 and 6 patients. Three-years LC, DMFS and OS rate were 59%, 51% and 76% respectively. At statistical analysis, only deep-seated tumors were correlated with impaired DMFS (29 months vs NR, p=0.0043) and OS (29 months vs NR, p=0.001), while no correlation was found between outcome and previous irradiation.

Conclusion: BAS is characterized by poor outcomes, and may require treatment intensification with more extended surgery due to prior management and possible prognostic role of deep-seated tumor.

(P 310) PREOPERATIVE RADIOTHERAPY WITH HYPERTHERMIA IN RECURRENT AND RADIATION-INDUCED SARCOMAS: RESULTS OF A PHASE 2 CLINICAL TRIAL

Piotr L. Rutkowski, MD, PhD[1]; Mateusz Jacek Spalek, MD, PhD[2]; Aneta Borkowska, MD, PhD[1]; Michal Wagrodzki, MD, PhD[3]; Patricia Castaneda-Wysocka, MD[4], (1) Maria Sklodowska-Curie National Research Institute of Oncology, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw Poland, Warsaw, Mazowieckie, Poland, (2) Maria Sklodowska-Curie National Research Institute of Oncology, Department of Soft Tissue/Bone Sarcoma and Melanoma, Department of Radiotherapy I, Warsaw Poland , Warsaw, Mazowieckie, Poland, (3) Maria Sklodowska-Curie National Research Institute of Pathology, Warsaw Poland, Warsaw, Mazowieckie, Poland, (4) Maria Sklodowska-Curie National Research Institute of Oncology, Department of Radiology, Warsaw Poland, Warsaw, Mazowieckie, Poland

Objective: The role of perioperative treatment in radiation-induced and in-field recurrent sarcomas (RIS/IFRS) is unknown. Re-irradiation may be associated with a risk of significant late toxicity and is therefore rarely used. We hypothesized that the combination of preoperative or definitive 12x 3 Gy radiotherapy (RT) with or without integrated 3.5 Gy to 42 Gy boost combined with twice-weekly regional hyperthermia would not result in significant late toxicity in patients with RIS/IFRS.

Methods: We conducted a prospective, single-arm, phase 2 clinical trial NCT04398095. We enrolled patients with locally advanced or oligometastatic RIS/IFRS. Treatment consisted of three weeks of radiotherapy, four fractions per week, 3 or 3.5 Gy per fraction, combined with regional hyperthermia, followed by surgery or observation. The choice of boost or no-boost regimen was based on resectability as assessed by two oncological surgeons. The procedure would be considered tolerable if significant RT-related late adverse events (grade 3+ CTCAE 5.0) occurred in less than 20% of patients at 18 months after RT. We planned to enroll 20 patients based on Wilson's method for calculating confidence intervals.

Results: We recruited 20 patients. Eight of them had RIS while twelve were diagnosed with IFRS. Median follow-up was 24 months. Mild or moderate radiation dermatitis was observed in 14 patients. One patient experienced grade 2 gastrointestinal toxicity. Late toxicities included limited limb mobility in two patients (grade 1 and 2), grade 1 skin fibrosis in four patients, and chronic skin ulceration (grade 2) in two patients. None of the patients available for evaluation developed grade 3 or higher late toxicity. Seven patients who received the no-boost regimen experienced local failure. None of the patients who received the boost regimen developed local progression. Eight patients developed distant metastases. Four patients died.

Conclusion: Our results provide hypothesis-generating data suggesting that preoperative re-irradiation with hyperthermia is a safe treatment for RIS/IFRS. However, the local efficacy without dose escalation is unsatisfactory, suggesting its importance in the design of further clinical trials.

(P 311) ROBUST VMAT TREATMENT PLANNING FOR EXTREMITY SOFT TISSUE SARCOMAS

Joshua Barrus, M.S.,R.T.(T)[1]; Katelyn Fernando, M.S. R.T.(T)[1]; Mark Addington, M.S.,CMD.,R.T.(T)[1]; Nishele D. Lenards, PhD, CMD., R.T.(R)(T), FAAMD[2]; Ashley Hunzeker, M.S., CMD[3]; David Konieczkowski, MD, PhD[4], (1) The Ohio State University Wexner Medical Center, Columbus, OH, (2) University of Wisconsin La Crosse, La-Crosse, WI, (3) University of Wisconsin La Crosse, Stewarville, MN, (4) James Cancer Hospital, The Ohio State University, Columbus, OH

Objective: Volumetric modulated arc therapy (VMAT) is a frequently employed radiotherapy (RT) modality for extremity soft tissue sarcomas (eSTS). Prior studies have shown that significant tumor volume changes during treatment result in loss of target volume coverage with highly conformal techniques such as VMAT, but few solutions exist to these issues aside from adaptive replanning. Here, we describe a related but novel phenomenon in which relatively minor changes in surface volume contour can result in unexpected subcutaneous hotspots. This is of significant clinical concern given the known association between skin dose and major wound complications during preoperative RT for eSTS. By evaluating daily cone-beam CT (CBCT) images from thirteen eSTS patients treated with VMAT RT, we identify daily surface contour changes (range: 2 mm-15 mm, median: 8 mm) which are frequently below conventional adaptive replanning thresholds. When applied under experimental conditions, these external contour changes did not have major impacts on target volume coverage (range: 30.2%-91.2%, mean: 72.5%) but did result in unexpected hotspots of 125.8% on average (range: 110.0%-142.2%) in the subcutaneous tissues. To mitigate this issue, we develop a methodology for VMAT treatment planning using flash PTV and virtual bolus (VB) to produce robust treatment plans that are more resistant to target volume changes, surface contour changes, and setup uncertainties than conventional planning methods. With this methodology, robust plans were equivalent to standard plans at baseline, but, after incorporation of surface volume changes, both maintained target volume coverage ($p < 0.001$) and prevented development of subcutaneous hotspots ($p < 0.001$) better than standard plans. This method may facilitate development of robust VMAT treatment plans that minimize development of subcutaneous hotspots and preserve target volume coverage in the context of routine volumetric changes during pre-operative RT.

Methods: Thirteen eSTS patients with PTV's extending close to the skin surface were simulated supine with their affected lower extremity immobilized in a vac-loc bag. Organs at risk included ipsilateral bones, subcutaneous strip, joints, skin (5 mm rind), and uninvolved body areas.
Treatment cone-beam CT (CBCT) images were evaluated and the largest external volume change from the plan CT was measured. For a representative example patient, we developed verification plans with varying degrees of PTV cropping from the external contour (3 mm and 5 mm) and degrees of uniform artificial tissue expansion (0 mm, 5 mm, 10 mm, and 15 mm) to explore these parameters. For the entire study population, an evaluation PTV (PTV_3mm_Eval) for optimization was cropped 3 mm from the surface. A 7 mm expansion was created from PTV_3mm_Eval and was cropped within the 5 mm skin rind (PTV_7mm). A 2 cm bolus was created for planning (Bolus_2cm), which covered PTV_7mm at the surface and was assigned a Hounsfield Unit (HU) value of -400. A 1.5 cm expansion from PTV_7mm (PTV_Flash) was created to provide a region of flash around the evaluation PTV and was cropped 1 mm outside PTV_3mm_Eval (Figure 1).
Eclipse with Acuros XB version 16.1.0 was used with VMAT on a Varian Truebeam linear accelerator. Plans used the arc angles, collimator angles, and field sizes from each original

treatment plan, with adjustments made only to accommodate the VB and PTV_Flash as needed (Figure 2). Plans were normalized so that 100% of the prescription dose covered 95% of the PTV.
Initial plans were calculated on the original planning CT scan without any changes to external body contour. To create the verification plan dataset, the external body contour was expanded by 1.5 cm in the areas where the PTV_7mm was in the skin contour (External_15mm). Parts of External_15mm extending outside the original external contour were isolated (Res_Override_Tis) and assigned an HU value of –101, to model the expansion of the affected limb during treatment. A 5 mm skin rind was created from External_15mm for dose evaluation (Skin_New_5mm, Figure 3).
Initial and verification plans were created with virtual bolus (VB) and with no virtual bolus (NB). Initial plans were re-optimized using the optimization objectives from each patient's original plan. Virtual bolus planning objectives included the addition of a lower objective asking for 75% of the prescription dose to PTV_Flash to control hot spots upon tissue expansion. Plans with VB were optimized with the bolus linked in Eclipse. After optimization, the VB was removed from the plan, and the dose was re-calculated with the same MLC sequence and monitor units (MUs) to create the VB plan. The final VB plan was evaluated to ensure that the dose remained clinically acceptable after removing the bolus. After the initial VB and NB plans were created, the plans were recalculated using the verification dataset to see the change in dose after volume changes had occured.

Results: The maximum external contour change observed (15 mm) was applied to the study. Using a single patient from the cohort, with a PTV cropped 5 mm from the skin surface, we found no appreciable difference in hotspots regardless of external contour change (range: 107.8%-109.2%; Table 1). For a PTV cropped 3 mm from the surface, an increase in hotspot was observed after external contour change with apparent relationships to the degree of external contour change and the location in which hotspots were measured. The greatest hotspot (116.0%) occurred with 15 mm of external contour change and within a 15 mm external rind. These differences generally increased as external contour change increased and were more pronounced at depths closest to the original skin location; however, increased hotspots were observed with external contour changes as small as 5 mm (Table 1).
We investigated these effects in the overall cohort. Plans with VB better maintained V100% PTV coverage after external contour changes compared to NB ($p < 0.001$; Table 2). Without VB, external contour changes comparable to that seen on CBCT (15 mm) resulted in a mean V100% PTV coverage of 72.5% (range: 30.2%-91.2%), while those with VB and flash PTV resulted a mean V100% PTV coverage of 93.1% (range: 85.5%-97.4%) on verifications plans (Table 2).
Plans with VB were more robust with regard to hotspots after external contour changes compared to NB ($p < 0.001$; Table 3). Without VB, extremity contour changes resulted in the development of a mean difference in hotspot of 14.6% (range: –0.5%-31.0%) on verifications plans when compared to initial plans (Table 3). With VB and flash PTV there was no appreciable hotspot development on verification plans (mean: -0.47%, range: -1.6%-1.8%; Table 3; Figure 4). This increase in robustness was achieved without increasing hotspots on the initial plans (initial NB plan hotspot mean: 111.2%, range: 108.7%-114.1%; initial VB plan hotspot mean: 111.7%, range: 109.2%-114.1%; Table 4).

Conclusion: This study demonstrates, to our knowledge for the first time in eSTS, that use of virtual bolus and flash PTV not only preserves target coverage with the relatively large changes in extremity contour that are often observed in eSTS, but also mitigates hotspots that can develop with relatively minor changes in external contour. Limitations of this study include its small size and single-institution nature. It is also important to note that we also used artificially expanded limb contours as the basis for presented calculations. These expansions were

chosen to reflect the range of external contour changes actually observed on daily CBCT in the study populations, and the controlled nature of these artificial expansions allowed detailed investigation into the effect of various changes on the relevant plans. Overall, however, the results of this study suggest an important but heretofore under-recognized possible pitfall in VMAT planning in extremity sarcomas, as well as a feasible methodology to overcome it.

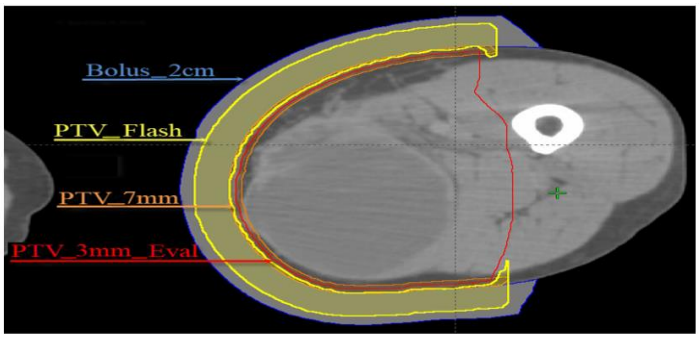


Figure 1. Planning Structures used for initial VB plans.

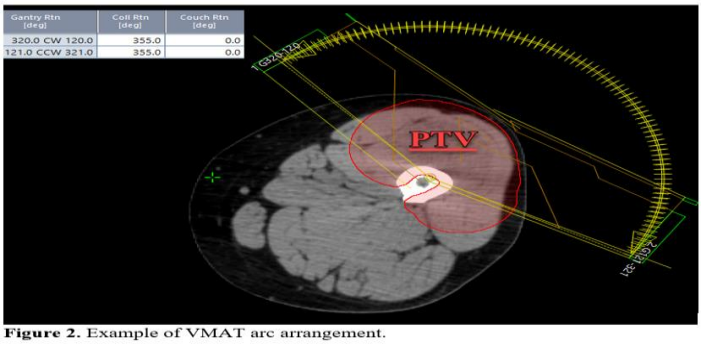


Figure 2. Example of VMAT arc arrangement.

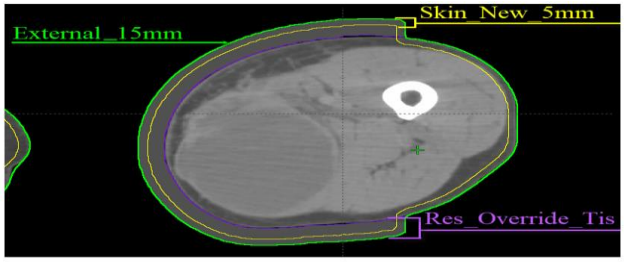


Figure 3. Structures created for verification plans to simulate 15 mm tissue expansion.

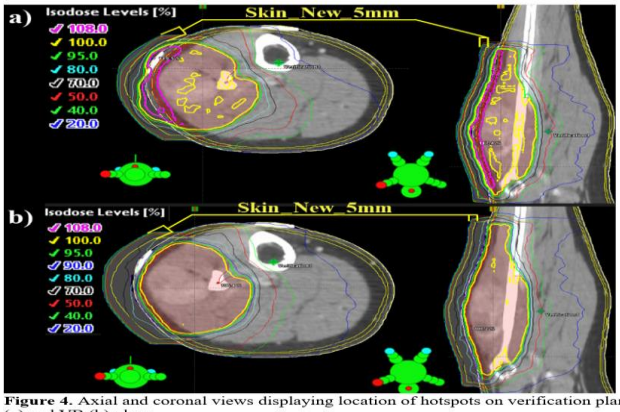


Figure 4. Axial and coronal views displaying location of hotspots on verification plans for NB (a) and VB (b) plans.

Table 1. Hotspots in skin rinds of varied thickness and external body contour expansions for 3mm and 5mm PTV external crop for a single patient.

PTV External Crop (mm)	**External Body Contour Expansion (mm)**	**Hotspot in External Rind**		
		5mm Rind	10mm Rind	15mm Rind
3	0	109.5%	109.7%	110.1%
	5	108.3%	113.8%	113.7%
	10	99.1%	112.2%	114.5%
	15	104.3%	114.4%	116.0%
5	0	105.1%	108.5%	109.1%
	5	96.5%	108.1%	108.1%
	10	87.9%	101.4%	107.8%
	15	98.0%	107.1%	109.2%

Table 2. Impact of virtual bolus on target volume coverage

	NB	**VB**	***t*-test**	**95% Confidence Interval of the Difference**	
	Mean		One-sided *P*-value	Lower	Upper
$V_{100\%}$ Coverage of PTV after external contour expansion	72.5%	93.1%	<0.001*	-31.0%	-10.2%
	Range				
	30.2%-91.2%	85.5%-97.4%			

* = *P*-value is statistically significant
NB = Non-virtual bolus plan; VB = Virtual bolus plan; V100% = Treatment volume receiving 100% of the dose

Table 3. Impact of virtual bolus on hotspots

	NB	**VB**	***t*-test**	**95% Confidence Interval of the Difference**	
	Mean		One-sided *P*-value	Lower	Upper
Increase in plan maximum dose following external contour expansion	14.6%	-0.469%	<0.001*	9.59%	20.6%
	Range				
	–0.5%-31.0%	-1.6%-1.8%			

* = *P*-value is statistically significant
NB = Non-virtual bolus plan; VB = Virtual bolus plan

Table 4. Summary of hotspots found in initial vs verification plans

	NB	**VB**
	Mean	
Plan Maximum Dose in Initial Plans	111.2%	111.7%
	Range	
	108.7%-114.1%	109.2%-114.1%
	Mean	
Plan Maximum Dose in Verification Plans	125.8%	111.2%
	Range	
	110.0%-142.2%	107.7%-115.9%

NB = Non-virtual bolus plan; VB = Virtual bolus plan

(P 312) MANAGEMENT OF ADULT HEAD AND NECK SOFT TISSUE SARCOMA: THE MAYO CLINIC EXPERIENCE

Will Sperduto, MD[1]; Molly M. Voss, BA[2]; Christopher Dodoo, MS[1]; Jessica Burlile, MD[3]; Omran Saifi, MD[4]; Brady Laughlin, MD[1]; Diego Santos Toesca, MD[1]; David Routman, MD[3]; Michelle Neben-Wittich, MD[3]; Byron C. May, MD[4]; Michael Rutenberg, MD, PhD[4]; Samir H. Patel, MD[1]; Safia K. Ahmed, MD[1], (1) Mayo Clinic Arizona, Phoenix, AZ, (2) Mayo Clinic Arizona, Scottsdale, AZ, (3) Mayo Clinic Minnesota, Rochester, MN, (4) Mayo Clinic Florida, Jacksonville, FL

Objective: Soft tissue sarcomas (STS) of the head and neck (H&N) are extremely rare, accounting for less than 1% of all STS. Given their rarity and heterogeneity, H&N STS are challenging entities to treat, requiring multidisciplinary collaboration. Herein, we report our institutional experience in the management of H&N STS.

Methods: An Institutional Review Board approved retrospective review of 97 adult patients with H&N STS treated with curative intent radiation therapy (RT) as part of management at Mayo Clinic (Minnesota, Arizona, and Florida) from 1998-2023 was performed. Inclusion criteria were age ≥ 18 years at diagnosis and disease localized to the H&N. Patient, tumor, treatment, toxicity, and outcomes data were gathered. Acute (during or up to 3 months from RT completion) and late (greater than 3 months after RT completion) toxicities were evaluated using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Kaplan Meier analysis was used to estimate local control (LC), progression-free survival (PFS), and overall survival (OS). Univariate and multivariate Cox regression analyses of disease and treatment characteristics were performed to identify predictors of LC, PFS, and OS. P-values less than 0.05 were statistically significant. All analyses were performed using R software.

Results: Table 1 lists patient, tumor, and treatment characteristics. Most common histologies included 23 (23.7%) angiosarcomas and 13 (13.4%) fibrosarcomas. Twenty-two patients had cutaneous angiosarcomas. Fifteen patients had RT-associated sarcomas. Thirty-seven (38.1%) patients received chemotherapy. Seventy-eight (80.4%) patients underwent surgery; 82% achieved R0 margins. Local recurrence rates by surgery versus no surgery were 15/78 (19.2%) and 9/19 (47.4%), respectively. Local recurrence rates by margin status were 9/62 (14.5%) R0, 4/8 (50%) R1, and 1/6 (16.7%) R2. Twenty-six (26.8%) patients received preoperative RT, 19 (19.6%) received definitive RT, and 52 (53.6%) received postoperative RT. Median total dose was 50 Gy in 25 fractions preoperatively, 60 Gy (IQR 50.4–66) in 33 fractions (IQR 28–35) definitively, and 60 Gy (IQR 59.9–64.8) in 30 fractions (IQR 30–33) postoperatively. Four patients received intraoperative RT. Acute and late grade 3+ toxicity rates were 20.6% and 19.6%, respectively. Most common acute and late grade 3+ toxicities were radiation dermatitis and mucositis, and skin ulceration and dysphagia, respectively. For patients with RT-associated sarcomas, acute and late grade 3+ toxicity rates were 0% and 26.7%, respectively. Median follow-up was 3.2 years (IQR 1.5–6.1). For the entire cohort, 3-year LC, PFS, and OS were 75.1% (66.2–85.1%), 54.2% (44.6–65.8), and 69.8% (60.6–80.3%), respectively (Figure 1). Three-year LC, PFS, and OS for the cutaneous angiosarcoma group were 53.7% (34.6–83.3%), 26% (12.6–53.6%), and 67.9% (50.8%–90.7%), respectively (Figure 2). Table 2 lists univariate analysis results. On multivariate analysis, tumor size [HR 1.18, 95% CI (1.04–1.34), $p = 0.009$] was an indicator for worse LC. Surgery was an indicator for better LC ($p = 0.036$) and OS ($p = 0.017$). Age at RT start [HR 1.04 (1.02–1.06), $p < 0.001$], N1 stage [HR 3.77 (1.55–9.13), $p = 0.003$], and tumor size [HR 1.19 (1.08–1.32), $p < 0.001$] were indicators for worse OS.

Conclusion: In one of the largest known studies of H&N sarcomas treated with RT, we report 3-year LC, PFS, and OS of 75.1%, 54.2%, and 69.8%, respectively. Larger tumor size was an indicator for worse LC. Surgery correlated with improved LC and OS. Older age, N1 stage, and larger tumor size were indicators for worse OS. Multidisciplinary treatment is imperative for this disease entity and local treatment intensification may be warranted for those at higher risk of local failure.

Table 1. Patient, tumor, and treatment characteristics

Characteristic	N (%)	Characteristic	N (%)
Sex		*TNM Stage[1]*	
Male	58 (59.8%)	T1	20 (20.6%)
Female	39 (40.2%)	T2	22 (22.7%)
Age		T3	31 (32%)
Median	68 years	T4a	13 (13.4%)
Range	18–92 years	T4b	2 (2.1%)
Race		N0	80 (90.9%)
White	87 (89.7%)	N1	8 (9.1%)
Other	10 (10.3%)	M0	88 (100%)
Size		M1	0 (0%)
Median	4.45 cm	*RMS TNM Stage[2]*	
Range	0.4–13.7 cm	T1a	1 (11.1%)
Grade		T1b	1 (11.1%)
1	8 (8.2%)	T2a	1 (11.1%)
2	10 (10.3%)	T2b	6 (66.7%)
3	78 (80.4%)	N0	4 (44.4%)
Not reported	1 (1%)	N1	5 (55.6%)
Primary Location		M0	9 (100%)
Neck	36 (37.1%)	M1	0 (0%)
Scalp / Face	33 (34%)	*RMS Clinical Group[2]*	
Nasal cavity / Paranasal sinuses	11 (11.3%)	I	3 (33.3%)
Oral cavity / Oropharynx	8 (8.2%)	II	1 (11.1%)
Salivary gland	5 (5.2%)	III	5 (55.6%)
Other	4 (4.1%)	*Margin Status*	
Histology		R0	62 (81.6%)
Angiosarcoma	23 (23.7%)	R1	8 (10.5%)
Fibrosarcoma	13 (13.4%)	R2	6 (7.9%)
UPS	11 (11.3%)	*RT Modality*	
Rhabdomyosarcoma	9 (9.3%)	Photons	62 (63.9%)
Leiomyosarcoma	7 (7.2%)	Protons	23 (23.7%)
Liposarcoma	7 (7.2%)	Electrons	11 (11.3%)
Synovial	6 (6.2%)	Chemotherapy	
MPNST	6 (6.2%)	Neoadjuvant	18 (48.6%)
Other	15 (15.5%)	Adjuvant	15 (40.5%)
Sarcoma Type		Both	4 (10.8%)
Cutaneous	22 (22.7%)	*Most Used Chemotherapy Agents*	
Soft tissue	75 (77.3%)	Doxorubicin, Ifosfamide, Cisplatin, Paclitaxel	

Grade based on FNCLCC. Tumors located in the 'Neck' predominantly originated from neck musculature and paraspinal or supraclavicular regions. Salivary gland = parotid (4) and submandibular (1). UPS = undifferentiated pleomorphic sarcoma. MPNST = malignant peripheral nerve sheath tumor. RMS = rhabdomyosarcoma. [1]American Joint Committee (AJCC) staging system for soft tissue sarcoma of the head and neck (8th ed, 2017). [2]Intergroup Rhabdomyosarcoma Study Group (IRSG) TNM (1997) and clinical group (1995) guidelines.

Variable	OS Hazard Ratio (95% CI)	OS P-value	LC Hazard Ratio (95% CI)	LC P-value
Age at RT Start	1.039 (1.017, 1.061)	0.001	1.031 (1.004, 1.058)	0.022
Sarcoma Type				
Cutaneous	Reference	—	Reference	—
Soft Tissue	0.66 (0.34, 1.28)	0.219	0.447 (0.195, 1.022)	0.056
Primary tumor size (largest dimension) [cm]	1.222 (1.096, 1.363)	<0.0001	1.225 (1.074, 1.399)	0.003
N Stage				
N0	Reference	—	Reference	—
N1	2.756 (1.335, 5.688)	0.006	1.508 (0.515, 4.419)	0.454
Chemotherapy				
No	Reference	—	Reference	—
Yes	1.534 (0.818, 2.875)	0.182	1.676 (0.751, 3.74)	0.207
Surgery				
No	Reference	—	Reference	—
Yes	0.173 (0.087, 0.346)	<0.0001	0.205 (0.087, 0.483)	<0.001
RT Modality/Type				
Photons	Reference	—	Reference	—
Electrons	0.676 (0.205, 2.235)	0.521	0.375 (0.05, 2.828)	0.341
Protons	1.796 (0.836, 3.861)	0.134	1.568 (0.634, 3.881)	0.33
Radiation timing				
Post-operative	Reference	—	Reference	—
Definitive	5.5 (2.599, 11.642)	<0.0001	5.796 (2.188, 15.357)	<0.001
Pre-operative	0.873 (0.375, 2.035)	0.753	1.539 (0.557, 4.246)	0.406

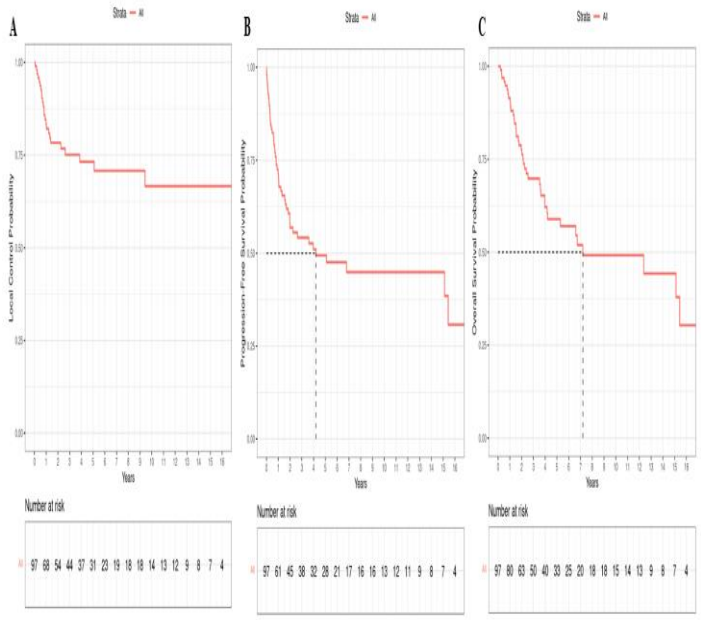


Figure 1. All patients. Kaplan Meier curves for (A) local control, (B) progression-free survival, and (C) overall survival.

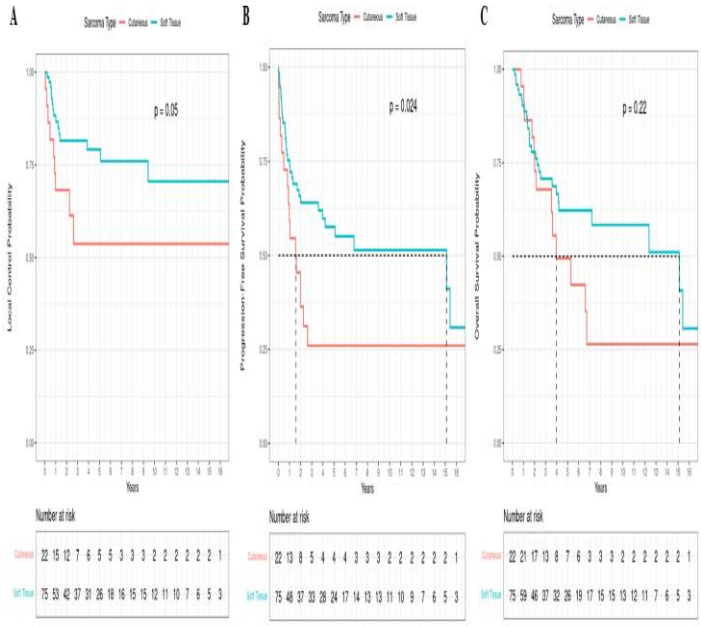


Figure 2. Cutaneous versus soft tissue sarcoma. Kaplan Meier curves for (A) local control, (B) progression-free survival, and (C) overall survival.

(P 313) SPACIALLY-FRACTIONATED RADIOTHERAPY (LATTICE) TREATMENT IN INOPERABLE NON-EXTREMITY BULKY SOFT-TISSUE SARCOMAS

Katarina Majercakova, MD[1]; Josep Isern Verdu, MD[1]; Antonio Vila Capel, MD[1]; Nagore Garcia Apellaniz, Medical Physicist[1]; José Antonio Gonzalez, MD, PhD[2]; Jady Rojas Cordero, MD[1]; Eugenia Otero Pla, MD[1]; Saba Rabi Mitre, MD[1]; Ana Sebio, MD, PhD[3]; Silvia Bague, MD, PhD[1]; Diana Hernandez Jover, MD[1]; Gemma Sancho Pardo, MD, PhD[1], (1) Hospital de la Santa Creu i Sant Pau, Barcelona, Catalonia, Spain, (2) Department of Surgery, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, Barcelona, Catalonia, Spain, (3) Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain., Barcelona, Catalonia, Spain

Objective: LATTICE is a technique of spatially-fractionated radiotherapy that allows to deliver a high dose of radiation (10-20Gy) to small volumes inside a tumor while simultaneously reducing the dose to normal tissue.

Our objective was to evaluate its feasibility, clinical outcome and toxicity in treatment of inoperable non-extremity soft-tissue sarcomas.

Methods: This retrospective observational study includes 9 histologically proven inoperable non-extremity sarcoma patients without option or no response to systemic treatment, treated between 2020-2022.

Six patients were treated with normofraccionated external beam radiotherapy (EBRT) 45-50 Gy (EQD2) and 3 patients with PS 2 were treated with short course treatment of 20-25Gy/5Gy per session. The VMAT technique was performed in all of them. A boost with Lattice technique of 20Gy single dose (8 patients) and 13.5Gy x 5 fractions (1 patient) in spheres, localized within the tumor was administered previously or after the end of standard EBRT.

Results: The mean follow-up since the beginning of the treatment was 10 months (4-23). 6 patients were male and 3 female. Mean age 55 years. Seven treated lesions were primary tumors, 2 metastatic lesions (1 pelvic and 1 ganglionar with multiple metastasis before the beginning of LATTICE treatment).

The tumor location was following: 1 mediastinum, 4 abdomen, 4 pelvis. The histology was: spindle cell sarcoma NOS (3), leiomyosarcoma (2), dedifferentiated liposarcoma(2), synovial sarcoma (1) and endometrial stromal sarcoma (1). The mean GTV volume was 1133 cm^3 (142-3580 cm^3). Mean number of spheres was 13 (4-30).

No acute and chronic G3-G5 toxicity was observed. Only 1 patient had G2 enteritis, due to 50 Gy + LATTICE 20 Gy. All 5 patients with symptoms before the treatment (dyspnea, pain, anemia, edema and constipation) presented symptom's relief.

Based on RECIST1.1 criteria, 8 patients (89%) had stable local disease. Only 1 patient (11%) with metastatic disease before the LATTICE had multiple progression including the local one at 3 months follow-up.

The surgery resection was feasible after the RT treatment in 3 patients (2 pelvic lesions and 1 bulky retroperitoneal sarcoma). 1 of them is alive without disease and 2 are dead due to metastatic progression.

3 patients are alive with stable disease (at 9,10 and 23 months of follow-up),1 patient with performed surgery is alive without disease and 5 patients are dead due to metastatic progression.

Conclusion: The LATTICE is a feasible radiation technique in inoperable bulky soft-tissue sarcomas without increment of the acute toxicity. It can be considered as a treatment option in bulky sarcomas for symptomatic relief and in some cases in the preoperative scenario.

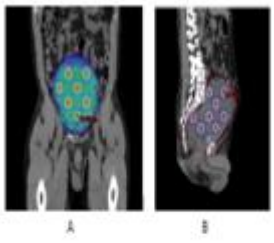


Figure 1. Example of Lattice planification in retroperitoneal sarcoma

A. Isodose of 45Gy/1.8Gy per fraction
B. Isodose of 20 Gy LATTICE boost

(P 314) PRE-OPERATIVE RADIATION THERAPY INCREASES ADHERENCE IN PATIENTS WITH HIGH-RISK EXTREMITY SOFT TISSUE SARCOMA

Hayley M. Dunlop, MPH[1]; TJ Atchison, BA[1]; Ryan Zeh, MD[2]; David Konieczkowski, MD, PhD[3]; Alex Kim, MD, PhD[4]; Valerie P. Grignol, MD[4]; Carlo M. Contreras, MD[4]; Samilia Obeng-Gyasi, MD[4]; Timothy M. Pawlik, MD, PhD, MPH[4]; Raphael E. Pollock, MD, PhD[4]; Joal D. Beane, MD[5], (1) The Ohio State University College of Medicine, Columbus, OH, (2) The University of Pittsburgh, Pittsburgh, PA, (3) James Cancer Hospital, The Ohio State University, Columbus, OH, (4) The James Cancer Hospital / The Ohio State University, Columbus, OH, (5) The James Cancer Hospital / The Ohio State University, Worthington, OH

Objective: Based on guidelines from the National Comprehensive Cancer Network (NCCN), treatment for extremity soft tissue sarcoma (ESTS) should include radiation therapy (RT) and surgical resection with negative margins for tumors that are high grade and >5 cm. Radiation has been demonstrated to reduce the risk of local recurrence and amputation for ESTS and, in a few studies, cancer specific survival. The recommended dose for pre-operative radiation therapy is 50 Gy while the recommended dose for post-operative radiation therapy is 60 Gy. Patients receiving less than the recommended doses of RT are at greater risk of local recurrence. RT is time and labor intensive for patients and social determinants of health may affect adherence, which may lead to poor patient outcomes. Whether sequencing of radiation therapy for patients with ESTS can be leveraged to improve compliance remains unknown. Therefore, the aim of this study was to define factors associated with completion of RT and to determine if pre-operative RT improved adherence compared to post-operative RT.

Methods: The analytic cohort was restricted to patients from 2004 to 2020 in the National Cancer Database with high grade ESTS >5 cm without nodal or distant metastases who

received limb-sparing surgery and RT with R0 margins. A multivariable logistic regression analysis identified factors associated with RT adherence defined according to NCCN guidelines. Variables in this model included radiation sequence (pre-operative, post-operative), distance from treating facility, facility type, facility region, age at diagnosis, primary payor, race, ethnicity, resection (local excision, radical resection), median income, and tumor size. A multivariable Cox Proportional Hazards model was used to assess overall survival. Independent variables in this model included radiation sequence (pre-operative, post-operative), distance from treating facility, facility type, facility region, age at diagnosis, primary payor, race, ethnicity, resection (local excision, radical resection), median income, tumor size, chemotherapy, histologic subtype, year of diagnosis, and Charlson-Deyo Comorbidity Index.

Results: Among 2327 patients, 47.7% received pre-operative RT (n=1111) and 51.9% (n=1207) received post-operative RT. A greater proportion of patients treated with pre-operative RT (51.9%) received the recommended dose of RT versus patients treated with post-operative RT (48.1%, n=787, p< 0.0001). Patients with private insurance (50.4% vs. 49.6%, p=0.027) and individuals treated at an academic medical center (53.9% vs. 46.1%, p< 0.001) were more likely to receive pre-operative RT. On multivariable analysis, patients who received pre-operative RT had lower odds of receiving an insufficient dose of RT (OR 0.34 [95% CI 0.26-0.45]). No differences in radiation therapy sequencing were observed by age, sex, income, education, urban/rural residence, or comorbidities (all p>0.05). Neither RT adherence nor radiation sequencing (post-operative RT vs. pre-operative RT) were independent predictors of overall survival.

Conclusion: There are multiple potential explanations for lower adherence to post-operative RT as compared to pre-operative RT. First, post-operative RT is a longer course of therapy, 6-6.5 weeks as opposed to 5 weeks, and therefore poses more substantial access and logistical barriers than pre-operative RT. Second, the higher dose of RT and larger field sizes required for post-operative RT, as compared with pre-operative RT, also creates more acute side effects (radiation dermatitis, acute edema, pain, etc.) that could motivate premature treatment discontinuation. There also may be a temptation for patients in a post-operative RT scenario to forego any further post-surgical treatment. Patients with large, high-grade ESTS who receive RT in the pre-operative RT setting are more likely to complete therapy and receive an optimal dose of RT compared to post-operative RT. The use of pre-operative RT improves adherence and should be widely considered in patients with high-grade ESTS, particularly in patients at risk for not completing therapy.

(P 315) SCALP VOLUMETRIC MODULATED ARC THERAPY USING 3D MILLED BOLUS: DOSIMITRY, TOXICITY AND OUTCOMES

Khaled Dibs, MD[1]; John Grecula, MD[1]; Darrion Mitchell, MD[2]; Sujith Baliga, MD[2]; Emile Gogineni, DO[2]; Sachin R. Jhawar, MD[2]; Wesley Zoller, CMD[2]; Mauricio Gamez, MD[3]; Dukagjin Blakaj, MD, PhD[2]; David Konieczkowski, MD, PhD[4], (1) Ohio State University Wexner Medical Center, Columbus, OH, (2) The Ohio State University Wexner Medical Center, Columbus, OH, (3) Mayo Clinic, Rochester, MN, (4) James Cancer Hospital, The Ohio State University, Columbus, OH

Objective: Radiotherapy for scalp lesions is frequently challenging due to the competing needs for target volume coverage, sparing of numerous critical nearby OARs, and reproducible bolus setup despite the irregular and highly convex scalp geometry. Here, we evaluate the use of 3D

milled bolus in treating such tumors.

Methods: A retrospective analysis of scalp patients (pts) treated between 2016-2022 using 3D milled bolus and Volumetric Modulated Arc Therapy (VMAT). All pts were treated with 6 MV photon beams, IGRT via daily CBCT, and 6-degree of Freedom (6-DOF) couch tops. Patient demographics, tumor characteristics, dose volume histograms (DVHs), toxicities and outcomes were evaluated. Regional control defined as control of the disease in the rest of the scalp/neck and local control defined as disease control within the PTV

Results: Eight pts were identified. Median age 74 (60-77), ECOG performance status was 0-1 in 7 pts (88%) and 88% were males. 38% of patients were treated with definitive intent to gross disease; the remaining 62% were treated post-operatively, 13% with microscopically positive margins and 50% with negative margins. 50% had T3/T4 and 88% had N0 disease. Median radiation dose was 66 Gy (60-69.96Gy). 50% received concurrent systemic therapy with Paclitaxel. In 63% of pts, the whole scalp was treated; in the remaining 37%, the median ratio of PTV volume to scalp volume was 60% (30-90%). 25% of patients (n=2) had neck irradiation for prophylactic (1pt)/ neck lymph node involvement (1 pt). The median homogeneity index was 1.07 (1.04-1.11). The median brain Dmax was 65 Gy (56-71.9), median brain mean dose was 22Gy (6-38.4), median eye Dmax was 11Gy (01.7-49), median cochlea mean was 8.4Gy (0.8-20.4), median lacrimal gland mean dose was 8.3Gy (0.11-38), median hippocampus Dmin was 6.5Gy (0.1-14.9) and Dmax was 12Gy (0.3-23). Most common acute side effects were grade 1-2 fatigue (100%), grade 1 pain (75%), grade 1 dysgeusia (25%), grade 1-2 dermatitis (63%). The only grade >=3 adverse event was grade 3 dermatitis in 37%. 25% developed grade 1 memory impairment. 25% had grade 1 eye dryness. None developed brain radionecrosis. At a median follow-up was 25 months (7-75), the 2-year locoregional control was 100%. 2-year DM control was 57%. 2-year OS was 70%.

Conclusion: VMAT planning with 3D milled bolus permits technically sound radiotherapy for scalp targets with an acceptable toxicity profile and relatively favorable clinical outcomes. This approach warrants further evaluation in a larger prospective study.

(P 316) THE ROLE OF RADIOTHERAPY IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS: A MULTICENTER COHORT STUDY

Christianne Jansma, Bsc[1]; J. Henk Coert, MD, PhD[2]; Dirk J. Grünhagen, MD, PhD[3]; Cornelis Verhoef, MD, PhD[3]; Enrico Martin, MD, PhD[2]; Walter Taal, MD, PhD[3], (1) Erasmus Medical Center and UMC Utrecht, Amsterdam, Noord-Holland, Netherlands, (2) UMC Utrecht, Utrecht, Utrecht, Netherlands, (3) Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands

Objective: Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are rare and aggressive malignant soft-tissue sarcomas (STS), of which 40% are associated with neurofibromatosis type 1 (NF1). Current treatment in localized disease mainly revolves around surgical excision, yet MPNSTs harbor a high probability of local recurrence (LR). Radiotherapy (RT) is increasingly being used, as it improves local control in STS, yet does not impact survival. Nevertheless, its use is controversial in some cases as major wound complications can be much higher and NF1 patients harbor a potential higher risk for secondary malignancies as MPNST commonly arise within plexiform neurofibromas. This study evaluates the use of radiotherapy in these rare tumors and tries to understand its impact in local control, especially

in the NF1 setting.

Methods: Surgically treated primary MPNSTs between 1988 to 2019 in the MONACO multicenter cohort were included. Demographic and treatment differences, especially the use of RT, between NF1 and non-NF1 were analyzed. Univariate and multivariable logistic regression analyses were used to identify factors independently associated with the use of radiotherapy. Multivariate Cox regression analyses were performed to identify factors associated with LR in NF1 patients.

Results: A total of 516 patients (32.6% NF1) were included, of which 149 (28.9%) developed a LR. RT was administered in 54.5% of patients (56.0% in NF1). Of these, 26.0% received neoadjuvant RT, and 79.2% adjuvant RT. The use of both treatment modalities was similar between patients with and without NF1. Multivariable regression showed that a high-grade tumor was the only independent factor for the use of radiotherapy. The rate of RT use in NF1 did not alter after correcting for tumor-, patient- and surgical factors. RT did not impact overall survival in sporadic and NF1 associated MPNST. In NF1 patients, microscopically positive margins (R1) (HR 2.2; 95% CI, 1.21-3.91) was the only independent risk factor for the development of a LR. Correcting for tumor-, patient- and surgical factors, use of RT was not associated lower rates of LR, in contrast to the sporadic population.

Conclusion: RT is commonly used in MPNST treatment regardless of its origin in NF1. While it may impact LR rate in sporadic patients, this is less clear in NF1 patients. Further studies are needed to evaluate its role and indications in NF1 associated MPNST.

Table. Multivariable logistic regression on factors associated with the use of radiotherapy

Variable	OR (95% CI)	p Value
NF1		
No	1.00	-
Yes	0.056 (-0.462 - 0.345)	0.777
Size		
<5 cm	1.00	-
>5 cm	0.345 (-0.116 – 0.806)	0.1416
Surgical margin		
R0	1.00	-
R1	0.319 (-0.121 – 0.759)	0.155
Depth		
Superficial	1.00	-
Deep	0.144 (-0.363 – 0.651)	0.574
Tumor grade		
Low grade	1.00	-
High grade	0.908 (0.197 – 1.62)	0.014

Table. Multivariate analysis of risk factors for the development of a local recurrence in NF1 patients		
Variable	**HR (95% CI)**	**p Value**
Grade		
Low grade	1.00	-
High grade	1.57 (0.465 – 5.72)	0.478
Size		
<5 cm	1.00	-
>5 cm	1.42 (0.564 – 3.55)	0.465
Radiotherapy primary tumor		
No	1.00	-
Yes	1.00 (0.557 – 1.81)	0.991
Depth		
Superficial	1.00	-
Deep	0.807 (0.359 – 1.81)	0.608
Margin primary tumor		
R0	1.00	-
R1	2.18 (1.21 – 3.91)	0.013

(P 317) PROGNOSTIC FACTORS, CLINICAL AND TOXICITY OUTCOMES FROM A LARGE COHORT OF PATIENTS WITH SOFT TISSUE SARCOMA TREATED WITH PREOPERATIVE RADIATION THERAPY

Mauro Loi, MD[1]; Daniela Greto, MD[1]; Marco Banini, MD[2]; Domenico Andrea A. Campanacci, Full Professor[3]; Guido Scoccianti, MD[3]; Ilaria Bonaparte, MD[2]; Giuliana Roselli, MD[4]; Annarita Palomba, MD[5]; Gabriele Simontacchi, MD[6]; Lorenzo Livi, Full Professor[2], (1) Radiation Oncology Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy., Florence, Toscana, Italy, (2) Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy, Florence, Toscana, Italy, (3) Department of Orthopaedic Oncology and Reconstructive Surgery, Azienda Ospedaliero Universitaria Careggi, Firenze, Italy, Florence, Toscana, Italy, (4) Department of Radiology, Azienda Ospedaliero Universitaria Careggi, Florence, Italy, Florence, Toscana, Italy, (5) Unit of Histopathology and Molecular Diagnostics, Careggi University Hospital, Florence, Italy., Florence, Toscana, Italy, (6) Radiotherapy Unit,Azienda Ospedaliero Universitaria Careggi, Florence, Toscana, Italy

Objective: Primary therapy for Soft Tissue Sarcoma (STS) often consists of a combined surgery and radiation therapy (RT). Preoperative RT may be advantageous due to lower dose requirements and possible tumor downstaging, particularly in association with chemotherapy (CT) and modern radiation techniques. This study evaluated efficacy, toxicity and prognostic-related features in STS patients treated with preoperative RT and surgery.

Methods: Clinical and treatment related data were collected from consecutive STS patients treated at our Institution with preoperative RT followed by surgery from 1991 to 2023. Overall survival (OS), Distant-metastasis free survival (DMFS), Local control (LC) data were collected and estimated using Kaplan-Meier method. Log-rank text and Cox model were conducted to

identify factors related with outcome and toxicity.

Results: 156 patients, for whom follow-up data were available, were included in our analysis. Median age was 55 years. Tumors were mostly located in the limbs (94%). Disease stage was T>=3 in 71% of cases. Most represented histotypes were liposarcoma (LPS) (34%), undifferentiated pleomorphic sarcoma (UPS) (24%) and synovial sarcoma (5%). RT prescription dose was 50 Gy in 25 daily fractions combined with CT in 82,5 % patients. IMRT was used in 34% of case. R0 and R1 resection were obtained in 93% and 7% of patients respectively. Severe surgical sequelae (wound dehiscence or sepsis) were reported in 16% of patients. Grade (G) 3 disease was observed in 69,8% patients. After a median follow-up of 32 months, OS rates at 2 and 5 years were 85% and 72%, DMFS rates were 69% and 57% and LC rates were 87% and 82%, respectively. At multivariate analysis only R1 resection resulted significant for worse LC rates (p=0.0214), whereas G3 disease was correlated with worse DMFS (p=0.0029). At Cox analysis G3 (p=0.004), UPS (p=0.0028) and R1 (p=0.05) correlated with worse overall survival. Use of IMRT and chemotherapy didn't result in higher severe surgical sequelae.

Conclusion: In this large STS cohort, neoadjuvant treatment resulted in high RO rate and favorable outcomes. R1 resection and G3 disease correlated respectively with impaired LC and DM FS. Both factors were associated with poorer OS, highlighting the interest of treatment intensification. IMRT and concurrent CT were not correlated with surgical sequelae.

(P 318) DEVELOPMENT OF NOVEL OUTLINING GUIDELINES FOR NORMAL TISSUE STRUCTURES FOR LIMB SOFT TISSUE SARCOMA.

Rita Simoes, PhD candidate[1]; Punita Shah, n/a[2]; Yolanda Augustin, PhD[3]; sarah Gulliford, PhD[4]; Peter Hoskin, MD[5]; kevin Harrington, PhD[6]; Aisha B. Miah, MD[3], (1) The Royal Marsden hospital, ICR, UCLH, RTTQA, London, England, United Kingdom, (2) The Royal Marsden hospital, London, England, United Kingdom, (3) The Royal Marsden Hospital, London, England, United Kingdom, (4) University College London Hospitals, London, England, United Kingdom, (5) University of Manchester, Manchester, England, United Kingdom, (6) The Institute of Cancer research, London, England, United Kingdom

Objective: RT to soft tissue sarcoma of the extremities (STSE) improves local control rates. However, STSE survivors often develop significant toxicities (defined as ≥ grade 2 RTOG; grade 2+), which impact on quality-of-life. Past clinical trials reported grade 2+ subcutaneous fibrosis (48.2% vs 31.5%), lymphoedema (23.2% vs 15.5%), bone fractures (11% vs 5%) and joint stiffness (23.2% vs 17.8%) associated with post-operative and pre-operative RT, respectively. Traditionally, RT plans are optimised to achieve maximum target coverage, whilst avoiding high doses to weight-bearing bones and intermediate to high doses to the normal tissue (NT) limb corridor. Novel NT outlining guidelines and atlas were developed, hypothesising that using these for RT planning will reduce RT toxicity. This study aims to examine the interobserver variations in lower limb NT outlining following the development of this novel approach.

Methods: Two STSE cases were selected as training cases: Case 1 - myxoid fibrosarcoma of the thigh and knee; case 2 - malignant peripheral nerve sheath tumour of the thigh. Target volumes had previously been outlined as part of their treatment courses. A set of reference

volumes was outlined on each case by one radiation therapist/therapeutic radiographer (RTT) and peer-reviewed by a consultant radiation oncologist specializing in STSE. NTs were then outlined by 7 additional observers (5 RTT and 2 clinicians) following the novel guidelines and atlas and re-outlined 6 months later by the reference volume expert to test intra-observer variability. The novel NT for STSE completed as part of this work were the muscle compartments of thigh (MCT), the neurovascular bundle (NVB), joints and bones (figure 1). Dice coefficients (DICE) were calculated for individual NT volumes against the gold-standard. The Kruskal-Wallis test was performed to detect statistically significant differences in DICE between NT volumes. A preliminary analysis of DICE was conducted after 3 observers (1 RTT; 2 clinicians) completed the cases and an amendment to the guidelines was carried out.

Results: Figure 2 shows the boxplot for DICE results above and below. There was a good agreement for the anterior, posterior, gluteal and adductor MCT, femur, femoral NVB and knee. Although there were differences in the DICE scores between RTTs and clinicians, these were not statistically significant (p=0.1). Differences in DICE were found for the lateral rotator MCT (p=0.008) which were corrected after training programme and guidelines refinement. Significant statistical (p=0.008) differences have been highlighted in the deep thigh and sciatic NVB and further corrections to guidelines to improve these 2 structures are currently under discussion.

Conclusion: Our results identify that novel NT outlining guidelines for STSE are reproducible between observers and, in a multi-professional environment, RTT and clinician scores are consistent. This work has also identified areas of refinement and modification required to improve the guidelines, particularly for the deep thigh and sciatic NVBs. Future work will enable us to attain dose-volume constraints for these structures which will also result in further refinements of the guidelines depending on significant areas to apply dose-volume constraints.

The first author is clinical doctoral research fellow CDR-2018-04-ST2-004, funded by HEE/ NIHR.

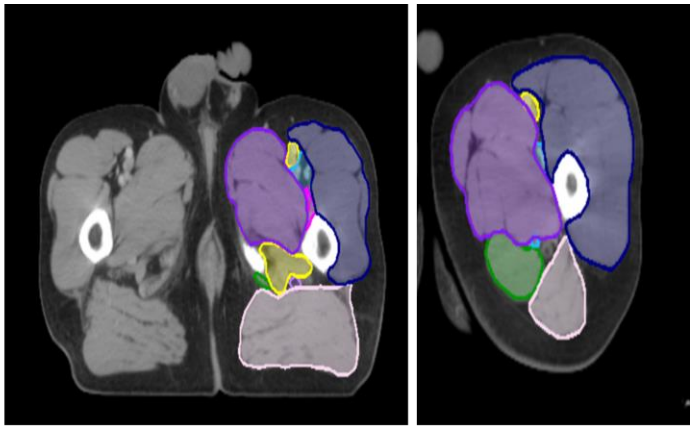


Figure 1. Outlining developed for STSE highlighting the anterior Muscle compartment (MCT_Anterior) in navy, the posterior anterior compartment (MCT_Posterior) in green, the adductor muscle compartment (MCT_Adductor) in purple, the lateral rotator muscle compartment (MCT_LatRotator) in yellow, the femoral neurovascular bundle (NVB_femoral) in yellow and deep thigh neurovascular bundle (NVB_DeepThigh).

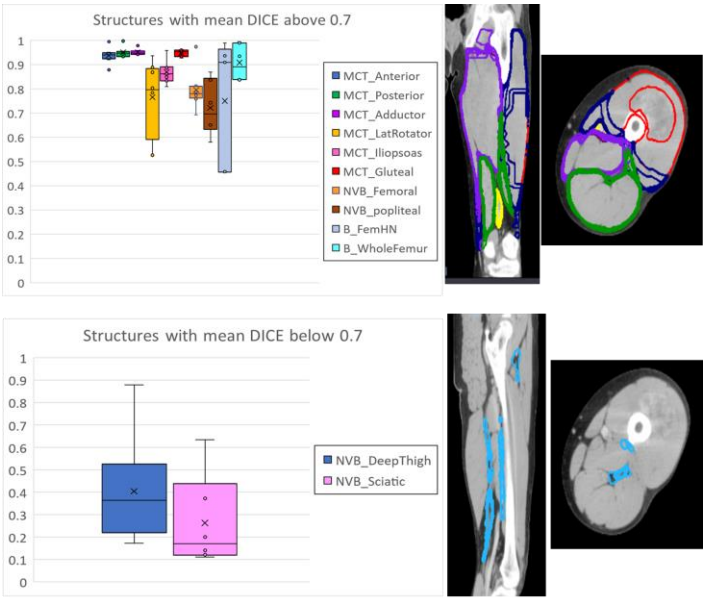


Figure 2. Mean dice coefficient boxplots for the normal tissue structures. Top pictures highlight structures with mean dice above 0.7 and bottom pictures mean dice coefficients below 0.7.

(P 319) HYPO-FRACTIONATED RADIATION THERAPY FOR THE EXTREMITY SOFT TISSUE SARCOM IN ELDERLY AND DISABLED.

Ran Dan Lin, MD[1]; Amir Sternheim, MD[2]; Viacheslave Soyfer, MD[2]; Ofer merimsky, MD[3]; Omri Merose, MD[2]; Solomon Dadia, MD[2]; Yair Gortzak, MD MSc[4]; Ortal Segal, MD[4], (1) Tel Aviv Soursky medical center, Kfar Saba, HaMerkaz, Israel, (2) Tel Aviv Medical Center, Tel Aviv, Israel, TEL AVIV, Tel Aviv, Israel, (3) oferm@tlvmc.gov.il, TEL AVIV, Tel Aviv, Israel, (4) Tel Aviv Medical Center, Tel Aviv, Israel, TEL AVIV, HaMerkaz, Israel

Objective: Introduction: The standards in treatment of soft tissue sarcoma are continuously evolving. The role of radiation therapy (RT) in reducing local recurrence is well established. According to the "Executive Summary of an ASTRO Clinical Practice Guideline" , neo-adjuvant RT (NART) is preferable as opposite to postoperative RT (PORT). The recently published phase 2 study of hypo-fractionated NART showed compatible results to standard radiation in terms of toxicity with similar survival. Elderly or disabled patients are unable to participate in the standard RT protocol due to its length. We have previously published our results of hypo fractionated PORT in elderly patients. The shifting paradigm in replacement of RT before the surgery was applied to this category of patients in our center. We, herein report on results of RT (mostly NART) in last decade.

Methods: Twenty consecutive patients with high grade soft tissue sarcoma of the extremities were treated in our tertiary center for soft-tissue sarcoma between the years 2015-2019. This is a retrospective EMR chart review looking at demographics, oncologic outcomes and complications.

Results: The median age of patients was 80.2 (62-92). Male/female ratio was 14/6. Anatomic

location was upper extremity in 35% and lower extremity in 65%. All cases were treated by hypo-fractionated doses of 39-45 Gy provided in 13-15 fractions. Nine patients were treated by NART and 11 by PORT. Mild to moderate acute toxicity developed in 2 patients (22.2%) in NART and in 1 patient (9%) in PORT. Significant wound complications including infection, dehiscence, re-hospitalization and surgery occurred in 4 (44.5%) patients in NART and 2 (18%) patients in PORT. Long term complications such as fibrosis and joint stiffness developed in 2 (22.2%) patients who received NART and 1(9%) patient in PORT. Local recurrence was developed in 3 patients in NART and in 2 in PORT treatment. The disease free survival was 30.3 (2-81), 30.8 (3-72) and 29.8 (2-81) month in all cohort, NART or PORT, respectively. The median survival was 38.7 (4-81), 38.3(16-72) and 39 (4-81) in all cohort, NART and PORT accordingly.

Conclusion: The standards Radiotherapy treatment of soft tissue sarcoma is hard to attain in the elderly and disabled patients. Short course radiation therapy, neo-adjuvant or adjuvant radiation therapy for soft tissue sarcoma in elderly or disabled patients achieves similar rates of acute and long term toxicity in our study, compared to historical data. The decreased reserves in elderly patients should always be considered in selecting the timing and fractionation of the treatment provided to the patient. The relatively high incidence of local recurrence observed in patients with initially worst prognostic factors (in 2 patients treated for local recurrence after previous surgery, and one patient who was diagnosed with aggressive angiosarcoma). The DFS and median survival in our study are not compromised. Our study supports results of recently published research, which shows the efficacy of preoperative hypo-fractionated radiotherapy in soft tissue sarcoma. The use of short NART in the treatment of STS in elderly patients. Should be encouraged after careful assessment of risk factors.

(P 320) A PROSPECTIVE MULTICENTER EXPERIENCE OF PROTON THERAPY FOR RHABDOMYOSARCOMA AND EWING SARCOMA: OUTCOMES FROM THE PROTON COLLABORATIVE GROUP REGISTRY

Matthew D. Hall, MD, MBA[1]; Katherine Von Werne, MSN, APRN[2]; Nicole Eiseler, MD[2]; Keitrina Mair, MS[2]; Noah S. Kalman, MD[2]; Ramon Jimenez, MD[2]; Giovanni Paraliticci, MD[2]; John Han-Chih Chang, MD[3]; Tamara Vern-Gross, N/A, DO[4]; Stephen A. Mihalcik, MD, PhD[5]; Young Kwok, MD[6]; Henry Tsai, MD[7]; Jing Zeng, MD[8]; Ronny Rotondo, MD[9]; Suzanne Wolden, MD[10]; Doured Daghistani, MD[2]; Juan Pretell-Mazzini, MD[2], (1) Miami Cancer Institute, Baptist Health South Florida, Miami, FL, (2) Miami Cancer Institute, Miami, FL, (3) ProCure, Oklahoma City, OK, (4) Mayo Clinic, Scottsdale, AZ, (5) Northwestern Medicine, Warrenville, IL, (6) University of Maryland, Baltimore, MD, (7) ProCure New Jersey, Princeton, NJ, (8) University of Seattle, Seattle, WA, (9) Kansas City Medical Center, Kansas City, KS, (10) Memorial Sloan Kettering Cancer Center, New York, NY

Objective: To report on the use of dose-escalated proton therapy (PT), acute toxicities, and outcomes in rhabdomyosarcoma and Ewing sarcoma patients enrolled on a prospective multi-institutional registry (PCG).

Methods: Data on patients with rhabdomyosarcoma and Ewing sarcoma treated with definitive-intent PT (defined as ≥45 Gy) were queried from the PCG registry. A similar query of our institutional database was performed with IRB approval. Overall survival rates were calculated

by Kaplan-Meier. Toxicities were scored using CTCAE v4.0.

Results: 354 patients across 10 institutions (203 rhabdomyosarcoma, 151 Ewing sarcoma) met the eligibility criteria. Median age was 9 years (Interquartile Range: 5-15). Median dose was 50.4 GyRBE for rhabdomyosarcoma patients (Range: 45-66 GyRBE) and 55.8 GyRBE for Ewing sarcoma patients (Range: 45-66 GyRBE). Median follow-up was 2.4 years (Range 0.3-12.3 years). Two-year overall survival rates were 81.1% (95% CI: 73.7%-88.5%) for rhabdomyosarcoma and 79.1% (95% CI: 71.7%-86.2%) for Ewing sarcoma. The Figure illustrates the prescription doses delivered by tumor histology; 28.1% of rhabdomyosarcoma and 21.9% of Ewing sarcoma patients, respectively, received dose-escalated radiotherapy (defined as >50.4 Gy for rhabdomyosarcoma and >55.8 Gy for Ewing sarcoma). Excluding alopecia and skin desquamation, 153 patients (43.2%) developed any acute grade 2+ non-hematologic toxicity, while 49 patients (13.8%) developed one or more grade 3 toxicities, as shown in the Table. The most common grade 3 toxicities were anorexia/weight loss (7.3%), head/neck mucositis, (4.8%), and nausea/vomiting (3.1%). One grade 4 toxicity (esophagitis) and no deaths were reported during treatment.

Conclusion: In this multi-institutional prospective registry, 28.1% of rhabdomyosarcoma and 21.9% of Ewing sarcoma patients received dose-escalated PT, with 13.8% of patients developing grade 3 toxicities. Long-term outcomes for disease control and late toxicity and anticipated cooperative group trial results are needed to fully assess the benefits and risks of dose-escalated radiotherapy for these tumors.

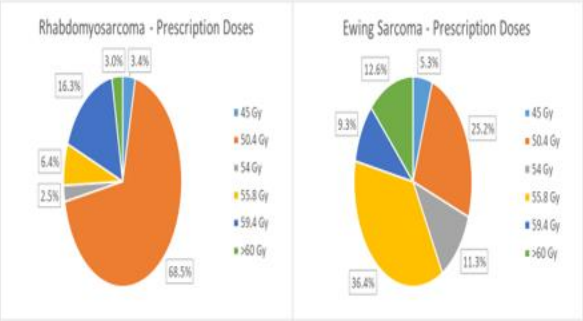


Table: Acute Toxicities during Proton Therapy

	Grade 2+	Grade 3+
Skin Desquamation	155 (43.8%)	27 (7.6%)
Fatigue	39 (11.0%)	1 (0.3%)
Anorexia/Weight Loss	26 (7.3%)	26 (7.3%)
Pain	26 (7.3%)	4 (1.1%)
Nausea/Vomiting	22 (6.2%)	11 (3.1%)
Head/Neck	43 (12.1%)	17 (4.8%)
Thorax	13 (3.7%)	8 (2.3%)
GI	13 (3.7%)	4 (1.1%)
GU	9 (2.5%)	0 (0%)
Gyn	1 (0.3%)	0 (0%)
CNS/Neuro	9 (2.5%)	2 (0.6%)
Musculoskeletal	0 (0%)	0 (0%)
Any Grade 2 or 3 Non-Dermatitis, Non-Alopecia Toxicity	**153 (43.2%)**	**49 (13.8%)**

(P 321) IDENTIFYING RISK FACTORS FOR REDUCTION OF PROTON THERAPY RE-PLANS FOR PAEDIATRIC PATIENTS WITH PELVIC SARCOMAS

Emma k. Austin, MBBS[1]; Jennifer Gains, MBBS MRCP FRCR MD (re)[2]; Pei Lim, n/a[2]; Yen Chin Chang, n/a[2]; Mark N. Gaze, MD FRCR FRCP[3], (1) University College Hospital London, London, England, United Kingdom, (2) University College Hospitals London, London, England, United Kingdom, (3) University College London Hospitals NHS Foundation Trust, London, England, United Kingdom

Objective: Proton beam therapy (PBT) is a precise form of radiotherapy used as treatment for local control for paediatric pelvic sarcomas. PBT is very sensitive to changes in anatomy and tissue density which can affect dosimetry resulting in under-coverage of the target volume or over-dosage of critical organs. Strategies such as image guidance, dosimetric assessment and replanning during treatment is necessary to ensure safe and effective delivery of PBT. The replanning process takes about 1-2 weeks and may therefore cause delays or gaps during PBT treatment.
We reviewed the replanning rates and risk factors for patients with pelvic sarcomas treated at our institution.

Methods: 13 pelvic sarcoma patients < 18 years treated between December 2021 to April 2023 were identified from our institutional database. Patients' medical records were reviewed and analysed for the reasons and timing of replans, weight charts, dietetic records and overall treatment time.

Results: Demographics are described in Table 1. The median age was 14 years (range 1-15). The cohort comprised of patients with Ewings (n=9), Rhabdomyosarcoma (n=3) and synovial sarcoma (n=1). Patients received doses of preoperative (n=3), definitive (n=9) or post operative PBT (n=1) given over 5 ½ to 6 weeks.
5 patients (38.5%) required replanning. 4 patients required one replan, these were due to separation gain identified on the cone beam CT (CBCT) on Fraction 1 (Fr1) of PBT. The separation gain varied from 0.6 – 1.2cm, resulting in the clinical target volume (CTV) being under dosed. The replans were initiated at week 1 (n=2), week 2 (n=1) and week 3 (n=1). 1 patient required two replans, in week 1 and 4. These were due to tumour shrinkage, causing small bowel to exceed recommended dose tolerance.
The mean weight change from planning CT scan (pCT) to Fr1 was 2.48kg in the replan group vs. 1.28kg in the non-replan group (p=0.2). The mean weight change from pCT to replan CT was higher at 2.98kg/5.88% of total body weight (range 1.3-4.4kg/2.9-10.5%) in the replan group (Table 2).
Dietician input occurred in 3 patients (60%) in the replan group after the weight gain was observed, in week 1 (n=2) and week 3 (n=1). After dietician input their weight varied by a maximum of 1.3kg, 1.2kg, 0.9kg during the remainder of their PBT compared 2.2kg, 1.3kg, 3.1kg prior to dietician input respectively.
Dietician input in the non-replan group occurred in 4 patients (50%), 3 of these saw a dietician prior to starting PBT. This was prompted by clinician due to fluctuations with weight during chemotherapy.
The were no unexpected treatment breaks and the length of treatment time was maintained in the replan group.

Conclusion: This review shows that weight changes are often seen alongside contour changes identified on CBCT. The majority of patients required a replan due to changes seen before the start of PBT. Weight gain of as little as 2.9% resulted in the need for a replan, highlighting the

importance of maintaining weight between the pCT and starting PBT as well as throughout the treatment course.
The planning process takes 3-4 weeks from pCT to starting PBT, ideally this interval would be reduced but due to the complexity of PBT planning and timing of chemotherapy this is often not possible.
37.5% of patients in the non-replan group v's 0% in the replan group had met a dietitian prior to the start of PBT. Our results suggest that early engagement of a dietician during the PBT planning process may be crucial in reducing replans due to proactive patient education and monitoring. We are working towards implementing dietician reviews at the first PBT clinic appointment for our patients and twice weekly weights. This will be re-audited after implementation to assess if this reduces weight changes and the number of replans.
Future work is being undertaken to explore the impact of weight changes from chemotherapy such as hydration regimens and utilisation of steroids prior to and during PBT.

Table 1 - Demographics

	Non replan group (n=8)	Replan group (n=5)
Age range	1 – 15 years	7- 15 years
Gender		
Male	6	3
Female	2	2
Histology		
Ewings	4	5
Rhabdomyosarcoma	3	0
Synovial sarcoma	1	0
Site		
Left	4	2
Right	2	3
Central	2	0
Stage of disease		
Localised	7	2
Metastatic	1	3
Chemotherapy		
Yes	8	5
No	0	0
Radiotherapy Dose & Fractionation		
59.4Gy 30#		3
59.4Gy 33#	0	1
58.1Gy 28#	1	0
54Gy 30#	3	0
50.4Gy 28#	4	1

Table 2 – weight change in the replan group

Weight at planning CT (kg)	Weight at replan CT (kg)	Weight change (kg)	% change
29.5	32.6	+3.1	10.5
96.6	101	+4.4	4.6
41.5	43.7	+2.2	5.3
44.9	46.2	+1.3	2.9
50.8	53.9	+3.1	6.1

(P 322) STEREOTACTIC BODY RADIOTHERAPY IN METASTATIC SARCOMA

Christina L. Stragliotto, M.D, PhD[1]; Mattias Hedman, N/A, M.D, PhD[2], (1) Karolinska University Hospital, STockholm, Stockholms Lan, Sweden, (2) Karolinska University Hospital, 171 76 Stockholm, Stockholms Lan, Sweden

Objective: The aim of this study was to retrospectively review the results of stereotactic body radiotherapy (SBRT) in 44 metastatic patients diagnosed with primary sarcoma at the Karolinska University Hospital. between the years 2008 and 2022. Overall response rate, overall survival and side effects were analyzed in 44 patients with a total number of 84 metastases.

Methods: A retrospective review was performed in 44 individual patients at the Karolinska University Hospital diagnosed between 2008-2022. Overall response rate, overall survival and side effects were analyzed in 44 patients with a total number of 84 metastases. The prescribed doses to the CTV were analyzed and the total number of fractions. The overall response (CR, PR, SD=local control) and PD was studied in each specific lesion as well as overall survival and side effects in each patient.

Results: Prescribed doses ranged from 6-17 Gy in 1-8 fractions with total doses of 12-51 Gy. Overall response rate at 6 months (local control= CR, PR and SD) for each tumor was 90%. Thirty-five metastases showed CR. The treated lesions were localized mainly in the lungs and liver. Median follow up was 27 months (3-150) months. Thirteen patients (29,5%) were long-term survivors and were still alive at last follow-up. The side effects were usually mild-moderate but 3 patients had grade 3 pneumonitis and 4 patients had rib fractures. One patient had a lethal grade 5 hemorrhage from the lung. Compared to our previous study the patients reviewed in this study had significantly smaller tumors, the tumors were treated with higher doses and the median overall survival was longer, 29,5 months versus 26,3 months.

Conclusion: We conclude that SBRT is a relatively safe and effective alternative to surgery in patients with metastatic sarcoma, however the optimal dose-fractionation schemes for lung and liver metastasis remain to be established. The primary cause of failure in this patient group was the development of new metastases.

(P 323) PERIPHERAL NEUROPATHY FOLLOWING INTRA-OPERATIVE RADIOTHERAPY FOR EXTREMITY SOFT TISSUE SARCOMA: A SINGLE INSTITUTION EXPERIENCE

Rahul D. Barve, MD[1]; Douglas Martin, MD[2]; Joel Mayerson, MD[2]; David Konieczkowski, MD, PhD[3], (1) Memorial Sloan Kettering Cancer , Columbus, OH, (2) The Ohio State University Comprehensive Cancer Center, Columbus, OH, (3) James Cancer Hospital, The Ohio State University, Columbus, OH

Objective: Extremity soft tissue sarcomas (eSTS) frequently arise along critical/unresectable structures, leading to close or positive surgical margins. Close or positive surgical margins can raise concern for increased local recurrence risk, and methods to address this concern are of interest. Intra-operative radiation therapy (IORT) provides such a method by delivering a single fraction boost dose to the at-risk margin during surgery. Here, we sought to review treatment toxicity and outcomes for patients who received surgery with IORT for eSTS.

Methods: We performed a retrospective study of patients with eSTS treated between 2013-2018 with external beam radiation therapy (EBRT) and surgery with IORT. We analyzed clinicopathologic variables, outcomes, and toxicity according to CTCAE v4.0 guidelines.

Results: We identified 22 patients with extremity STS treated with surgery + IORT in the study timeframe. The lower extremity was involved in eighteen (82%) patients and the upper extremity in 4 (18%). Median age was 67 (range 35-88) and 41% were male. Ten patients (45%) patients had a > 10 pack years smoking history, and one (5%) had diabetes mellitus. None had previously received radiation therapy. Most common tumor histologies included pleomorphic sarcoma in 6 (27%), myxoid liposarcoma in 5 (23%), and myxoid fibrosarcoma in 4 (18%). All patients underwent surgical resection with IORT; of these, 16 (73%) received preoperative EBRT and 1 (5%) received post-operative EBRT. Thirteen (59%) patients received pre-op systemic therapy, most commonly with HD-AIM (40%); eight patients (36 %) received adjuvant chemotherapy, most commonly with pazopanib (18%). Surgical margins were microscopically negative (R0) in 18 patients (82%) and microscopically positive (R1) in 4 (18%). All patients received electron IORT with 6 MeV electrons; median IORT dose was 10 Gy (range 10-15). Eighteen patients (82%) had nerves in proximity or abutting the tumor that were included in the IORT field. Of these 18 patients, 6 (33%) developed grade 3 neuropathy (extremity weakness, foot drop, or use of AFO or cane). Neuropathy developed at a median of 10 months (range 7-12 months) after surgery+ IORT. All patients developing neuropathy had received pre-operative EBRT as well as chemotherapy. Mean BED was 131.9 vs 105.0 Gy in patients developing vs not developing neuropathy (p=0.0365). In all 6 patients, the nerve deficit was consistent with neuropathy arising in the IORT field. On follow up, 8 (36%) patients had distant recurrence in lungs and 1 (5%) patient had local recurrence requiring amputation. Sixteen (78%) patients were alive, five (23%) were dead, and one (5%) was lost to follow up.

Conclusion: Although the combination of surgery, and typically EBRT, with an IORT boost is an attractive approach in principle to address close or positive surgical margins in eSTS, this study describes a significant risk of neuropathy with this approach. Peripheral nerves are dose-limiting structures for IORT, and significant caution should be exercised in using IORT when major peripheral nerves are located within the treatment field.

(P 324) EVALUATING THE ROLE OF ADJUVANT RADIATION AFTER WIDE LOCAL EXCISION OF PLEOMORPHIC DERMAL SARCOMA

Sarah Yuen, MD MPH[1]; Yingjoy Li, BA[2]; James Jakowatz, MD[3]; Maki Yamamoto, MD[3]; Thuy Tran, MD[3], (1) University of California Irvine, Orange, CA, (2) UNIVERSITY OF CALIFORNIA IRVINE, Orange, CA, (3) UNIVERSITY OF CALIFORNIA IRVINE, ORANGE, CA

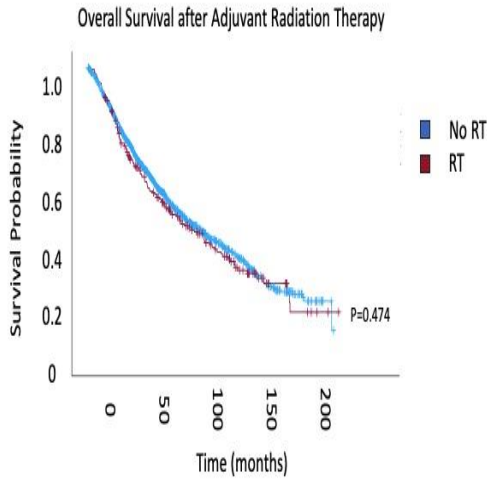
Objective: Pleomorphic dermal sarcomas (PDS) are rare undifferentiated mesenchymal tumors that are poorly defined but have adverse histological features and metastatic potential. Treatment is centered around surgical excision; however, the optimal adjuvant treatment regimen is unclear due to its rarity. This study aims to evaluate the impact of adjuvant radiation therapy (RT) on long-term survival in patients with PDS after surgical excision.

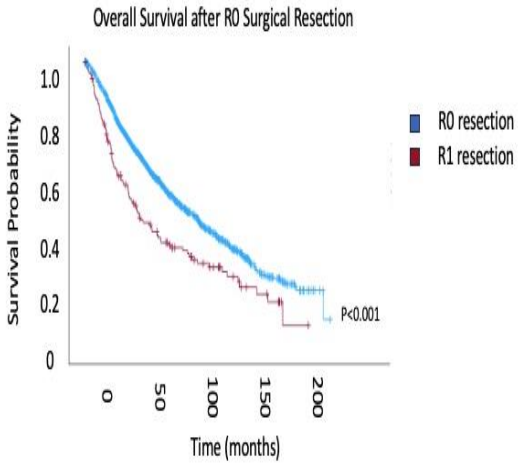
Methods: The National Cancer Database was queried to identify patients with PDS who underwent surgical excision. Patients were stratified based on receipt of RT. Demographics,

tumor characteristics and treatment type were evaluated. Kaplan Meier method was used to estimate overall survival (OS). Multivariable Cox proportional regression models were performed to identify predictors of OS.

Results: A total of 1442 patients were identified with PDS who underwent surgical excision, with 219 (15.2%) patients who received RT compared to 1223 (84.8%) patients who did not. There was no statistically significant difference in treatment facility type or location, patient gender, race, median household income, or comorbidity scores between the two groups based on receipt of RT or not. Regarding tumor characteristics, patients who received RT tended to have tumors greater than 2cm (58.6% vs 34.7%, P< 0.001), high grade tumors (28.3% vs 11.9%, P< 0.001), presence of lymphovascular invasion (LVI) (5.3% vs 0.7%, P< 0.001), positive regional lymph nodes (3.8% vs 0.4%,P< 0.001), and positive surgical margins (22.8% vs 8.1%, P< 0.001). Patients who received RT were also treated with chemotherapy (4.7% vs 0.7%, p< 0.001) more often than those who did not receive RT. The median OS was higher for patients with small tumors < 2cm compared to those with larger tumors >2cm (84.3 months vs. 41.4 months, P< 0.001). R0 margin status was also associated with significantly improved survival compared to those with positive margins after resection (median OS 82.3 months vs. 43.6 months, P< 0.001). However, adjuvant radiation was not associated with improved survival (median 78 months vs 71 months, P=0.474). Subgroup analysis of patients with R1 margin status after surgery showed no differences in survival with or without radiation (median OS 45 months vs. 41.3 months, p=0.998). On multivariate analysis, older age (HR 1.07, P< 0.001), high comorbidity score (HR 1.71, P=0.014), tumor size (HR 1.60, P< 0.001), and positive margin status (HR 1.68, P=0.001) were independent predictors of poor survival.

Conclusion: Adjuvant RT was not associated with improve survival in resected PDS, regardless of margin status. Due to limited effective systemic treatment options for pleomorphic dermal sarcoma, curative intent complete resection with R0 margins remains the cornerstone of treatment for PDS. While the use of RT in the treatment of PDS was more common in patients with tumors with more aggressive features, there remains limited data to support the use or omission of adjuvant RT in PDS. The role of adjuvant RT should be an area of future investigation, and adjuvant RT should be considered for PDS on a case-by-case basis based on local recurrence risk or potential risk of distant metastases.





(P 325) OUTCOMES AFTER MODERN THREE-DIMENSIONAL CONFORMAL RADIOTHERAPY AND INTENSITY MODULATED RADIOTHERAPY TO TREAT SARCOMAS ARISING IN THE HAND: A RETROSPECTIVE SERIES

Franel Le Grange, MBChB FCRadOnc MD[1]; Avinash Pilar, MBBS MD FRCR[2]; Mahbubl Ahmed, MRCP PhD FRCR[2]; Beatrice Seddon, MBBS BSc MRCP FRCR PhD[2], (1) UCLH, London, England, United Kingdom, (2) University College London Hospital, London, England, United Kingdom

Objective: Limb-salvage surgery and adjuvant radiotherapy to the hand-wrist complex pose challenges for surgeons and radiation therapists alike. Maintaining hand function is an important goal. The smaller soft tissue volumes around the hand-wrist complex may impact on the quality of radiation dose delivery and increase the risk of acute skin reaction. We explore the outcomes for patients treated with modern three-dimensional conformal radiotherapy (3DCRT) and intensity modulated radiotherapy (IMRT) in a large sarcoma referral centre.

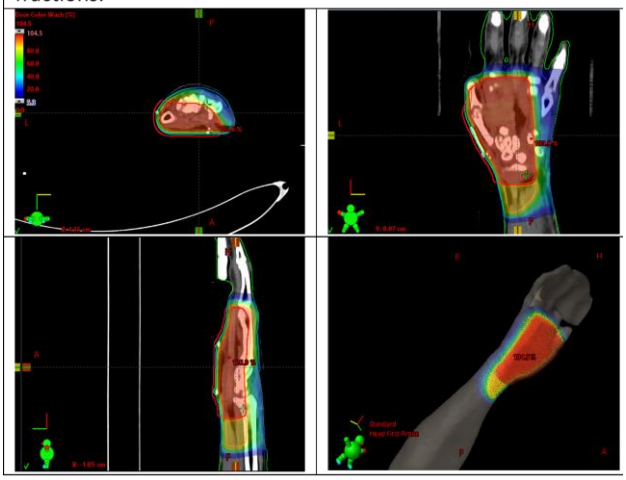
Methods: Adult patients with sarcoma of the hand-wrist area, treated with high dose radiotherapy, were retrospectively identified from sarcoma radiotherapy records dating back no further than 2001. Demographic data was collected on age, gender, site, histological subtype and grade, tumour size, and staging. Surgical data included procedure and resection margin status. Radiotherapy details included technique, dose-fractionation, timing of radiotherapy, treatment interruptions, acute and late effects. Outcome data was collected on local recurrence, distant metastases, and survival.

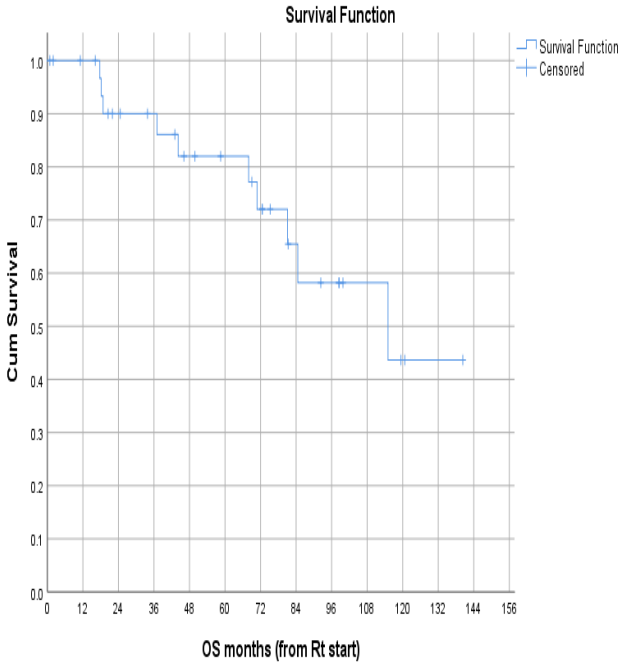
Results: 34 adult patients were treated between 2001-2023, median age 52 years(range 23-82), 52% male and 47% female. Tumours were centred on the hand(59%) or wrist(41%). 68% were newly diagnosed and 29% treated for local recurrence. 6% had metastases at the time of treatment. The most common subtypes were synovial sarcoma(29%), malignant peripheral nerve sheath tumour(12%), leiomyosarcoma(9%), myxofibrosarcoma(9%), myxoinflammatory fibroblastic sarcoma(9%), clear cell sarcoma(6%), spindle cell sarcoma(6%), and myoepithelioma(6%). Majority were grade 2 or 3(74%) and deep to fascia(85%). Median tumour size was 4.5cm(range 1.5-8cm). Surgery involved excision of finger rays or distal radius/ulna in

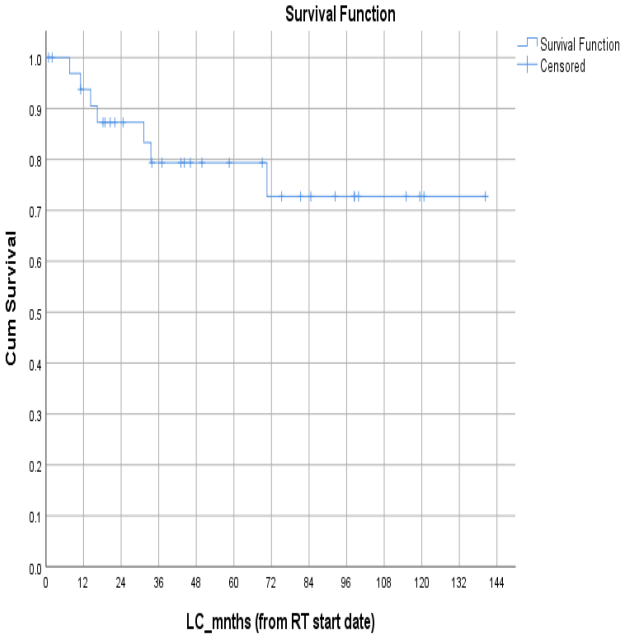
15% and 9%. Resection margins were R1 in 9% and R2 in 12%. R0 margins ranged from 1mm or less(44%) to wide. 9% had neo-adjuvant chemotherapy. Radiotherapy was pre-operative(24%) or post-operative(74%), and definitive in 1 patient. Treatment was with 3DCRT(79%), IMRT(18%) (example in Fig 1), and retreatment with brachytherapy in 1 case. Most frequent dose-fractionations were 60Gy/30 fractions(59%) and 50Gy/25 fractions(24%). Grade 3 skin reaction/swelling was described in 38%. 5 patients receiving 3DCRT had treatment interruption(6%) or did not complete the planned treatment(9%) due to acute skin reaction occurring after cumulative doses of 54Gy or more. All patients receiving IMRT completed treatment without interruption. Median follow up was 63 months(range 1-140). Outcomes at 5 and 10 years were: Overall survival 82% and 43.6% (Fig 2); Local control 79.3% and 72.7% (Fig 3); Distant metastases free survival 72.1% and 48.5% (Fig 4). Local recurrence was at median 16 months(range 8-71). Data on late effects and functional outcomes were not systematically collected in this retrospective series, but significant complications and functional loss were described in 6 patients(18%). Four of these had aggressive surgery with distal radial or ray resection.

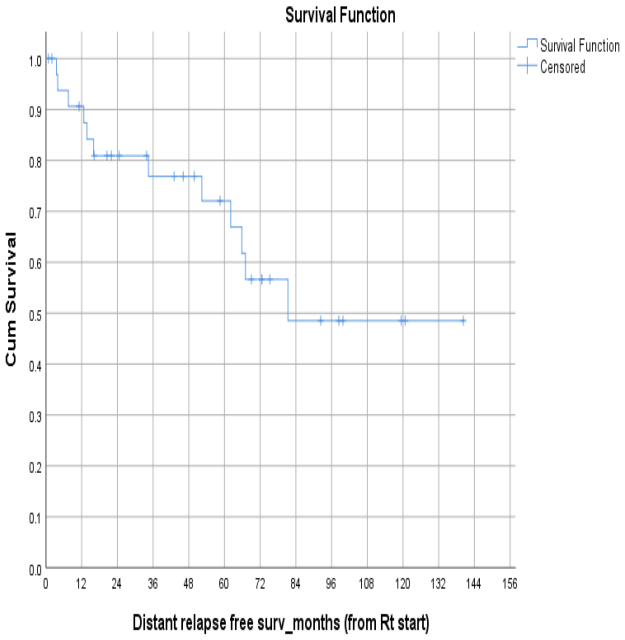
Conclusion: This is the largest series of radiotherapy to sarcomas of the hand and wrist treated exclusively in the modern era of 3DCRT, including patients treated with IMRT, a technique not previously reported in the literature for this site specifically. The 5-year local control and survival outcomes in this cohort were comparable to published series, supporting a limb salvage approach with a combination of surgery and radiotherapy in this high-risk population where resection was marginal or incomplete in most cases. The reduced 10-year distant metastases free and overall survival rates potentially reflect the spectrum of late relapsing histological subtypes represented in this cohort. Radiotherapy delivery was challenging with a high incidence of significant acute toxicity reported, and treatment was interrupted or stopped before it could be completed in 15% of cases, all in the 3DCRT group. None of these patients developed a local recurrence however, raising a question about the potential for dose or dose intensity reduction that could be explored in future research. Local control was achieved at the cost of long-term functional loss in 18%, in line with functional outcomes reported in other series.

Fig1: Example IMRT plan. Synovial sarcoma of the hand treated post-operatively, 2 dose levels: 60Gy and 52.2Gy in 30 fractions.




Survival Function
Survival Function
Censored
Cum Survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
12
24
36
48
60
72
84
96
108
120
132
144
156
OS months (from Rt start)


Survival Function
Survival Function
Censored
Cum Survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
12
24
36
48
60
72
84
96
108
120
132
144
LC_mnths (from RT start date)



Withdrawn ~~(P 326) **CARBON-ION RADIOTHERAPY FOR UNRESECTABLE PELVIC SARCOMAS/CHORDOMAS**~~

Retroperitoneal and Abdominal Sarcomas

(P 327) LEARNING CURVE ANALYSIS IN RETROPERITONEAL SARCOMA SURGERY

Laura Samà, MD[1]; Sonia Kumar, MD[1]; Laura Ruspi, MD[1]; Federico Sicoli, MD[1]; Vittoria D'Amato, Pharmacy[1]; Alice Laffi, MD[1]; Salvatore Lorenzo Renne, MD[1]; Davide Baldaccini, MD[1]; Elena Clerici, MD[2]; Alexia Francesca Bertuzzi, MD[3]; Pierina Navarria, MD[1]; Vittorio Lorenzo Quagliuolo, MD[1]; Ferdinando Carlo Maria Cananzi, MD[1], (1) IRCCS Humanitas Research Hospital, Rozzano, Milan, Lombardia, Italy, (2) Rozzano, Milan IRCCS Humanitas Research Hospital, Rozzano, Milan, Lombardia, Italy, (3) Humanitas Research Hospital, Rozzano, Lombardia, Italy

Objective: In retroperitoneal sarcoma (RPS) surgeons undergo a learning process to acquire the necessary skills for optimal patient outcomes. This study aimed to assess the relationship between surgical experience and operative outcomes and identify distinct phases and factors that influence the learning process in RPS surgery.

Methods: A retrospective analysis was conducted from a prospectively collected database. We analyzed 62 consecutive RPS surgeries performed by the same surgeon (Jan 2014-Jan 2023) at IRCCS Humanitas Research Hospital (Rozzano, Milan). We used the cumulative sum (CUSUM) analysis to assess the learning curve and identify significant changes in surgical

performance over time.

Results: The mean age of patients was 61.5 years (range 24-83). Male:female ratio was 27:35. Mean size of tumors was 174 (range 0-617, where we considered as 0 if no macroscopic residual disease was detected at histology). Forty-five cases (72.6%) were primary tumors or residual after incomplete resection, whereas 17 cases (27.4%) were local recurrences. Tumors were located in the left retroperitoneum (RP) in 24 patients (38.7%), in the right RP in 26 patients (42.0%), and in pelvis in 12 patients (19.3%). Histologic subtypes were well-differentiated liposarcoma (WDLPS,19.4%), dedifferentiated liposarcoma (DDLPS,53.2%), leiomyosarcoma (LMS,17.7%), other (9.7%). Forty-four cases (71.0%) were high-grade tumors (G2-G3). Neoadjuvant chemotherapy was performed in 18 cases (29.9%), while neoadjuvant radiotherapy in 10 cases (16.1%). The procedures included 47 (75.8%) multivisceral resections. Pattern of resection can be seen in Table 1. The mean operative time was 358 minutes (range 108-838), with a mean intraoperative blood loss of 590 ml (range 50-4000). The operative time (OT) learning curve was analyzed (Image 1B). The learning curve consisted of three unique phases: phase 1 (the 16 initial cases), phase 2 (the middle 30 cases), and phase 3 (the subsequent 16 cases). Phase 1 was characterized by a negative slope, which represented shorter operating time during this phase. Phase 2 was characterized by a plateau phase. Phase 3 was achieved after 46 cases and showed a positive slope that indicates longer operation times. Comparisons of various parameters between the three phases are presented in Table 2. Statistically significant difference between phase 1 and 2 combined and phase 3 was achieved by size (p=0.003), presentation (p=0.048), pattern of resection (colon+kidney+other, p=0.033), operation time (p=0.006), and length of stay (LOS) (p=0.026).

Conclusion: This study aimed to fill a critical knowledge gap by examining the learning curve in RPS surgery. The findings will contribute to enhance surgical training, optimizing patient outcomes, and to improve decision-making in this challenging surgical field. We selected CUSUM analysis due to the limitations of interpreting raw data plotted chronologically. The CUSUM graph provides insights into the variance from the mean on a case-by-case basis, resulting in a parabolic curve that exhibits three distinct phases. These phases enable the assessment of learning curve correlates. Phase 1 represented the initial learning curve. Phases 2 and 3 showed an increase in operative times that reflect the performance of more complex cases as surgeon competence increases. Understanding the learning curve in RPS surgery will help guide surgical education and refine surgical techniques, ultimately leading to improved patient care and long-term oncological outcomes.

Image 1: Surgeon operative time (OT). (A) OT plotted against case number. (B) Cumulative sum $(CUSUM)_{OT}$ plotted against case number (solid line). The dashed line represents the curve of best fit for the plot (a second-order polynomial).

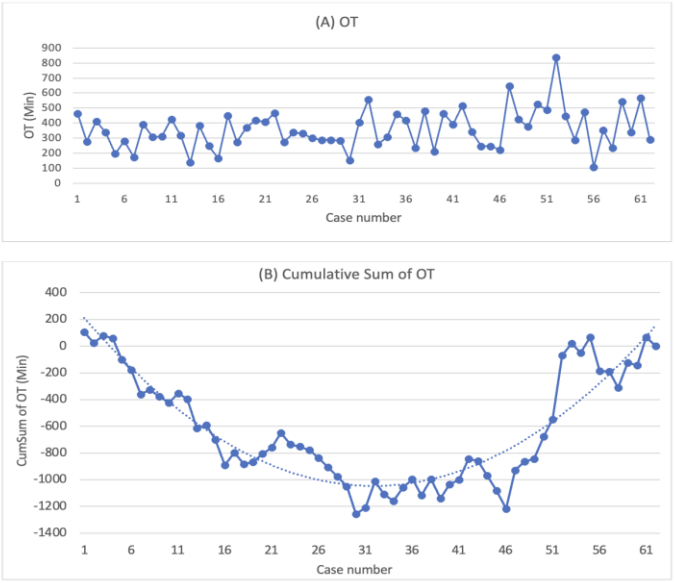


Table 1: Patient demographics, preoperative diagnoses, surgical procedures, operative characteristics, and postoperative outcomes

		N=62 (%)
Patients Characteristics	Mean age (range)	61.5 (24-83)
	M/F[1]	27 (43.5%)/35 (56.5%)
Tumor characteristics	Mean size (range)	174 (0-617)[2]
	Primary/Recurrence	45 (72.6%)/17 (27.4%)
	Site	
	• Left RP[3]	• 24 (38.7%)
	• Right RP	• 26 (42.0%)
	• Pelvis	• 12 (19.3%)
	Histology	
	• WDLPS[4]	• 12 (19.4%)
	• DDLPS[5]	• 33 (53.2%)
	• LMS[6]	• 11 (17.7%)
	• Other	• 6 (9.7%)
	G1/G2-G3[7]	18 (29.0%)/44 (71.0%)
	Neoadjuvant CT[8]	18 (29.9%)
	Neoadjuvant RT[9]	10 (16.1%)
Surgery	Multivisceral Resection	47 (75.8%)
	Pattern of organ resection	
	None	7 (11.3%)
	One	8 (12.9%)
	Multivisceral	47 (75.8%)
	• Colon±Kidney±Other	• 18 (38.3%)
	• Colon±Kidney±Spleen±Distal Pancreas±Other	• 9 (19.1%)
	• Vascular	• 10 (21.3%)
	Tumor Rupture	2 (3.2%)
	R2[10]	1 (1.6%)
	Mean OT[11] (range)	358 (108-838)
	Mean Intraoperative Blood Loss[12] (range)	590 (50-4000)
Surgical Outcomes	Complications	36 (58%)
	CDC >=3[13]	13 (37.1%)
	Mean LOS[14] (range)	17 (5-58)

[1] Male/Female
[2] Tumor size (in mm) 0= radicalization after inadequate surgery without macroscopical residual disease at histology
[3] Retroperitoneum
[4] Well-differentiated liposarcoma
[5] Dedifferentiated liposarcoma
[6] Leiomyosarcoma
[7] Grade
[8] Chemiotherapy.
[9] Radiotherapy
[10] R2= macroscopic residual disease after surgery
[11] Mean operative time in minutes
[12] in ml
[13] Postoperative complications classified with the Clavien-Dindo classification
[14] Mean length of stay in days

Table 2: Interphase comparisons of patient, tumor characteristics, intraoperative parameters, and short-term outcome

	Phase 1 n=16 (%)	Phase 2 n= 30 (%)	Phase 3 n= 16 (%)	Phases 1 and 2 versus phase 3 (*p*-value)
Mean age (range)	57 (24-79)	61 (35-83)	67 (46-83)	0.057
M/F[1]	6/10	14/16	7/9	0.985
Tumor Size (range)	136 (50- 350)	152 (0-50)[2]	254 (7.5-617)	**0.003**
Primary[3]/ Recurrence,	10 (62.5%)/6 (37.5%)	21 (70.0%)/9 (30.0%)	14 (87.5%)/2 (12.5%)	**0.048**
RP[4]/Pelvis	11 (67.7%)/ 5 (31.3%)	24 (80.0%)/ 6 (20.0%)	15 (93.8%)/1 (6.2%)	0.126
Left RP/ Right RP[5]	7 (63.6%)/4 (36.4%)	12 (50.0%)/12 (50.0%)	5 (33.3%)/10 (66.7%)	0.178
G1 /G2-G3[6]	3 (18.8%)/13 (81.2%)	9(30%)/21 (70%)	6 (37.5%)/10 (62.5%)	0.390
Neoadjuvant CT[7]	4 (25.0%)	8 (26.7%)	6 (37.5%)	0.390
Neoadjuvant RT[8]	1 (6.3%)	5 (16.7%)	4 (25.0%)	0.266
Multivisceral Resection	10 (62.5%)	23 (76.7%)	14 (87.5%)	0.216
Colon-Kidney	0 (0.0%)	10 (33.3%)	8 (50.0%)	**0.033**
Spleen-Distal Pancreas	3 (18.8%)	2 (6.7%)	4 (25%)	0.170
Vascular	1 (6.3%)	5 (16.7%)	4 (25%)	0.266
Tumor Rupture	0 (0.0%)	2 (6.7%)	0 (0.0%)	0.400
R2[9]	1 (6.3%)	0 (0.0%)	0 (0.0%)	0.555
Mean OT[10]	302 (range 138-463)	347 (range 151-558)	434 (range 108-838)	**0.006**
Intraoperative Blood Loss[11]	447 (range 50-1500)	548 (range50-2500)	813 (range50-4000)	0.163
CDC >=3[12]	1 (6.3%)	9 (30%)	3 (18.8%)	0.585
Mean LOS[13] (range)	12 (5-25)	16 (5-58)	23 (5-54)	**0.023**

[1] Male/Female
[2] Tumor size (in mm) 0= radicalization after inadequate surgery without macroscopical residual disease at histology
[3] Primary = primary or residual after incomplete resection
[4] Retroperitoneum
[5] N=50, we excluded pelvis
[6] Grade
[7] Chemotherapy
[8] Radiotherapy
[9] R2= macroscopic residual disease after surgery
[10] Mean operative time in minutes
[11] in ml
[12] Postoperative complications classified with the Clavien-Dindo classification
[13] Mean length of stay in days

(P 328) REAL-LIFE EXPERIENCE OF PAZOPANIB IN PATIENTS WITH ADVANCED UTERINE LEIOMYOSARCOMA (U-LMS) FROM TWO REFERRAL ITALIAN CENTERS

Chiara Fabbroni, MD[1]; Mara Mantiero, MD[2]; Monika Ducceschi, MD[3]; Marta Bini, Resident[3]; Maggie Polignano, MD[3]; Elena Fumagalli, MD[4]; Salvatore Lopez, MD, PhD[3]; Andrea Franza, Resident[3]; Francesco Raspagliesi, MD/Prof[5]; Gabriella Parma, MD[6]; Nicoletta Colombo, MD/Prof[7]; Paolo Giovanni G. Casali, MD[8]; Roberta Sanfilippo, MD[9], (1) Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (2) INT milano, milan, Lombardia, Italy, (3) INT Milan, milan, Lombardia, Italy, (4) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (5) INT, Milan, Lombardia, Italy, (6) IEO, milan, Lombardia, Italy, (7) IEO, Milan, Lombardia, Italy, (8) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (9) Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Lombardia, Italy

Objective: U-LMS is a rare entity that needs to be referred to highly specialized sarcoma centers. The antiangiogenic agent Pazopanib is an oral multitargeted tirosine-kinase inhibitor already approved for soft tissue sarcoma with a Progression Free Survival (PFS) of 3 months in a phase III randomized study (PALETTE trial).

Methods: We retrospectively investigated all cases of patients with advanced U-LMS treated with Pazopanib at two Italian referral centers since September 2013. We reported the real word-data focusing on PFS, responses and complicated responses.

Results: From September 2013 to May 2023, 38 women with metastatic U-LMS received Pazopanib. The majority of patients were PS ECOG 0 or 1 (36/38); the median age was 52 years old (38-72 years old). Twenty-two/38 (58%) patients had more than 1 metastatic site with

the most frequent one represented by lung (17/22). The median number of previous chemotherapy was 3 (range 2-5). All but 8 patients started Pazopanib at 800 mg daily; and ten of them (33%) required a dose reduction. Thirty-four patients were evaluable for response: 6/34 (18%) patients had a partial response, 12/34 (35%) patients had a SD. In the subgroup of patients treated at National Cancer Institute 7/26 (27%) patients had a response according to CHOI criteria. In the same subgroup, in 5/26 (19%) patients a complicated response was observed: in particular 1 pneumothorax, 2 intestinal perforation, 1 intestinal occlusion and 1 emoftoe due to pulmonary cavitation. In a further subgroup of 12 patients from the same center, we were able to evaluate hyperprogression that was observed in 7/12 (58%) patients. In the overall population, median PFS was 4.8 months.

Conclusion: In a real-world population of patients with advanced U-LMS treated with Pazopanib, the data of PFS and responses are consistent with literature. The variation of tumor density and CHOI criteria, should be considered in the evaluation of responses. However, clinicians must be careful during treatment due to the possibility of complicated response with the use of Pazopanib

(P 329) DISTANT METASTASIS IN RETROPERITONEAL LEIOMYOSARCOMA: TUMOR SIZE, GRADE AND MORE?

William W. Tseng, MD[1]; Francesco Barretta, PhD[2]; Marco Fiore, MD, FEBS, FACS[3]; Chiara Colombo, MD[4]; Stefano Radaelli, MD[5]; Marco Baia, MD[6]; Carlo Morosi, MD[7]; Paola Collini, MD[8]; Roberta Sanfilippo, MD[9]; Chiara Fabbroni, MD[10]; Silvia Stacchiotti, MD[11]; Dario Callegaro, MD[6]; Alessandro Gronchi, MD, FSSO[12], (1) City of Hope National Medical Center, Duarte, CA, (2) Fondazione IRCCS IStituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (3) Department of Surgery, Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (4) Department of Surgery, Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (5) Sarcoma Service, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (6) Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (7) Department of Radiology, Fondazione IRCSS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (8) Department of Advanced Diagnostics , Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (9) Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Lombardia, Italy, (10) Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (11) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (12) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy

Objective: Distant metastasis is the predominant issue in patients with retroperitoneal leiomyosarcoma (RP LMS) after complete resection. This is the rationale for including this histologic type in the ongoing STRASS2 phase 3 study, which randomizes retroperitoneal sarcoma patients to neoadjuvant chemotherapy followed by surgery versus surgery alone. We sought to determine variables associated with distant metastasis (DM) and disease-specific death (DSD) in RP LMS to apply clinical practice as well as STRASS2 trial analysis and future translational investigation.

Methods: Data were collected on patients with primary RP LMS who underwent surgery at the Fondazione IRCCS Istituto Nazionale dei Tumori in Milan, Italy, a high-volume specialist center,

from 2002-2023. Patients who had incomplete resection, recurrence or metastatic disease at presentation and gynecologic- or genitourinary-origin tumors were excluded. Descriptive data were summarized and crude cumulative incidence curves were estimated for DM and DSD in a competing risk setting. Univariable and multivariable Fine and Gray models were performed to assess the association of specific variables of interest (e.g., tumor size) on outcome.

Results: Our study included 157 patients with a 2:1 female to male ratio. Median tumor size was 11.0 cm and 96.2% of patients had an intermediate or high grade (FNCLCC 2 or 3) tumor. The tumor origin as determined at surgery and by pathologic examination of the resection specimen was inferior vena cava in 40.8% of patients, another major vessel (e.g., renal, iliac) in 18.5%, gonadal vein in 14.6% and unclear in 26.1%. All patients underwent complete resection. Blood transfusions were given at surgery in 46.5% of patients and the overall frequency of major complications was low (14.0%). Chemotherapy was given in 56.7% (43.9% neoadjuvant) and radiation therapy in 14.6% of patients. With a median follow-up of 3.8 years, the overall 5-year incidence of local recurrence was extremely low (2.4%) but was as high as 60.1% for DM. On univariable and multivariable models, only tumor size and grade were significantly associated with DM and DSD (all $p < 0.05$). No other variable, including site of tumor origin or chemotherapy given was found to have any association with these outcomes. Female gender was found to demonstrate a trend for lower incidences of DM ($p = 0.09$) and DSD ($p = 0.08$) on multivariable analysis. Five-year incidences of DM and DSD by tumor size and grade are shown in the Table. Post-hoc analysis of preselected patients with 1) DM less than 1 year from surgery or 2) no evidence of disease > 5 years confirmed that tumor size and grade are the only variables that significantly differ in these subgroups.

Conclusion: Among conventional clinicopathologic data, tumor size and grade are the only predictors of DM and DSD in patients with RP LMS. As data from the prospective STRASS2 trial emerge, these two variables can help to identify subgroups that are most impacted by neoadjuvant chemotherapy. Our study also highlights the critical unmet need for better biomarkers (e.g., in the tumor microenvironment or circulating blood) to stratify DM risk in RP LMS.

Table. Incidence of distant metastasis and disease-specific death by tumor size, grade

	DM	95% CI	p	DSD	95% CI	p
Tumor Size (cm)						
<11	46.9	(35.2-62.4)	0.004	19.4	(10.4-36.2)	0.019
≥11	72.7	(61.4-86.0)		35.0	(24.2-50.7)	
Grade						
2	48.7	(36.1-65.7)	0.003	11.2	(4.7-26.6)	<0.001
3	71.2	(60.4-84.0)		44.5	(32.2-61.4)	

(P 330) TREATMENT VARIATIONS, OUTCOMES AND PATTERNS OF RECURRENCE AFTER CURATIVE INTENT TREATMENT FOR PRIMARY RETROPERITONEAL SARCOMAS IN LATIN AMERICA. FIRST COLLABORATIVE LATAMSARC STUDY

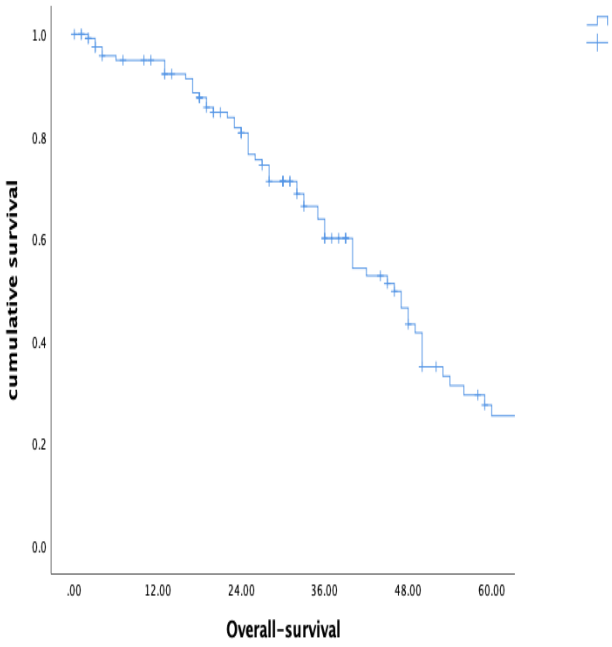
Dorian Yarih García-Ortega, MD, MSc, PhD, FACS[1]; Sergio D. Quildrian, MD[2]; Nicolas Devaud, MD[3]; Catherine Sarre-Lazcano, MD, MSc, MHA[4]; Diana Otero Norza, MD[5]; Paul Adriazola Vicente, MD[6], (1) Instituto Nacional de Cancerología (México), Tlalpan, Distrito Federal, Mexico, (2) University of Buenos Aires, Angel H. Roffo Institute of Oncology - Buenos Aires British Hospital, Buenos Aires, Ciudad Autonoma de Buenos Aires, Argentina, (3) Instituto Oncologico Fundacion Arturo Lopez Perez (FALP), Santiago, Region Metropolitana, Chile, (4) Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Toronto, ON, Canada, (5) Hospital Dr. Rafael Angel Calderón Guardia, San José, San Jose, Costa Rica, (6) Hospital Nacional Edgardo Rebagliati Martins - Universidad Ricardo Palma, Bellavista, Callao, Peru

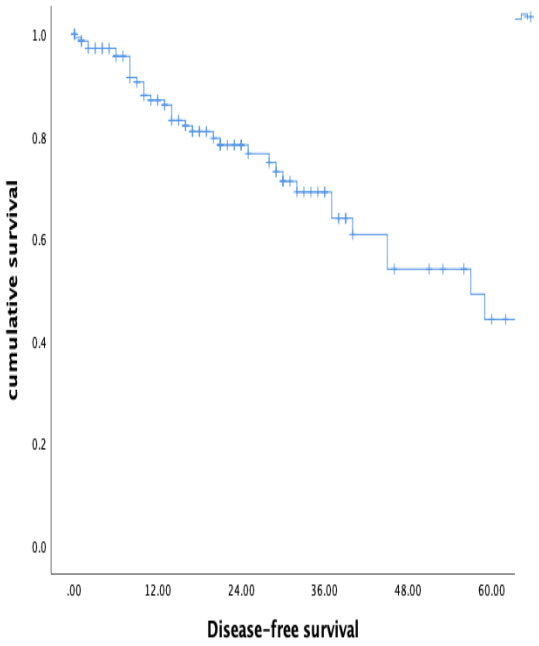
Objective: Retroperitoneal sarcomas (RPS) are rare tumors composed of several well-defined histologic subtypes. The objective of this study was to assess the epidemiology, treatment patterns, and outcomes of patients with retroperitoneal sarcoma in Latin America. The aim of this study was to analyze patterns of recurrence and treatment variations in a large population of patients treated at reference centers.

Methods: This retrospective descriptive study utilized an electronic database of medical records from LATAMSARC (a group studying sarcomas consisting of different centers in various countries in Latin America, including Argentina, Chile, Costa Rica, Mexico, and Peru). Adult patients (≥18 years) with retroperitoneal sarcoma who underwent surgery at six cancer centers in Latin America were included. Key outcomes were practice patterns, overall survival (OS), and progression-free survival (PFS).

Results: Three hundred patients with retroperitoneal sarcomas met the inclusion/exclusion criteria. The mean age was 53 years (SD 13.37), with 53% being male and 47% female. Out of these, 272 (90.7%) were located in the extrapelvic retroperitoneum, and 28 (9.3%) were in the pelvic retroperitoneum. The tumor size was 23.53 cm (SD 13.8 cm). The most common subtypes were well-differentiated liposarcoma (30.5%), dedifferentiated liposarcoma (24.6%), leiomyosarcoma (10.8%), malignant peripheral nerve sheath tumor (6.2%), solitary fibrous tumor (1.5%), and other or non-classifiable (8.4%). Compartmental resection was performed in 34.7% of cases, involved organ resection in 26.3%, simple resection in 32.7%, 6% were deemed unresectable, and 0.3% had missing data. Tumor rupture was reported in 15 cases (5%). The kidney was the most frequently resected organ, with 123 cases (41%), followed by the right colon in 52 cases (17.3%) and the left colon in 46 cases (15.3%). Complications with Clavien-Dindo scale >3 occurred in 56 cases (18.7%), with bleeding being the most frequent in 28 cases. Sixty-two patients required intensive care unit stay, the duration of surgery was 240 minutes (SD 131 minutes), and the hospital stay was 11.9 days (SD 9.6). The median follow-up was 26 months (Interquartile range: 11-39). Median overall survival (OS) was 52.20 months [95% confidence interval (CI): 44.95-59.44]. The local recurrence (LR) rate was 54.3 % with a median of 47.012 months (95% CI: 40.33, 53.69) in this cohort of patients.

Conclusion: This study provides information on the epidemiology, treatment patterns, and outcomes of retroperitoneal sarcomas in a group of reference centers in Latin America. These results represent the largest body of evidence on retroperitoneal sarcomas in Latin America.


cumulative survival
1.0
0.8
0.6
0.4
0.2
0.0
.00
12.00
24.00
36.00
48.00
60.00
Overall-survival


cumulative survival
1.0
0.8
0.6
0.4
0.2
0.0
.00
12.00
24.00
36.00
48.00
60.00
Disease-free survival

(P 331) MUSCULAR DEDIFFERENTIATION IN DEDIFFERENTIATED LIPOSARCOMA: INSIGHTS INTO INFLAMMATORY PROFILES USING THE NEUTROPHIL/LYMPHOCYTE RATIO AND PROGNOSTIC IMPLICATIONS

Dorian Yarih García-Ortega, MD, MSc, PhD, FACS[1]; Claudia Haydee Caro, MD[2]; Ana Paulina Melendez-Fernandez, MD[3]; Kuauhyama Luna-Ortiz, MD[1], (1) Instituto Nacional de Cancerología (México), Tlalpan, Distrito Federal, Mexico, (2) The National Cancer Institute, Cuidad de Mexico, Estado de México, Mexico, (3) Instituto Nacional de Cancerología (Mexico), Tlalpan, Distrito Federal, Mexico

Objective: Soft tissue sarcomas comprise a diverse group of rare malignancies, with 15% originating in the retroperitoneum. These tumors often present at an advanced stage, leading to lower disease-free survival (DFS) and overall survival (OS), with recurrence rates approaching 50% in high-volume centers and OS reaching up to 60%. Dedifferentiated liposarcomas (ddLPS) are aggressive neoplasms, and the presence of dedifferentiated components is associated with increased distant recurrence and reduced OS. However, the impact of specific dedifferentiation subtypes and their association with systemic inflammatory profiles remains poorly understood.

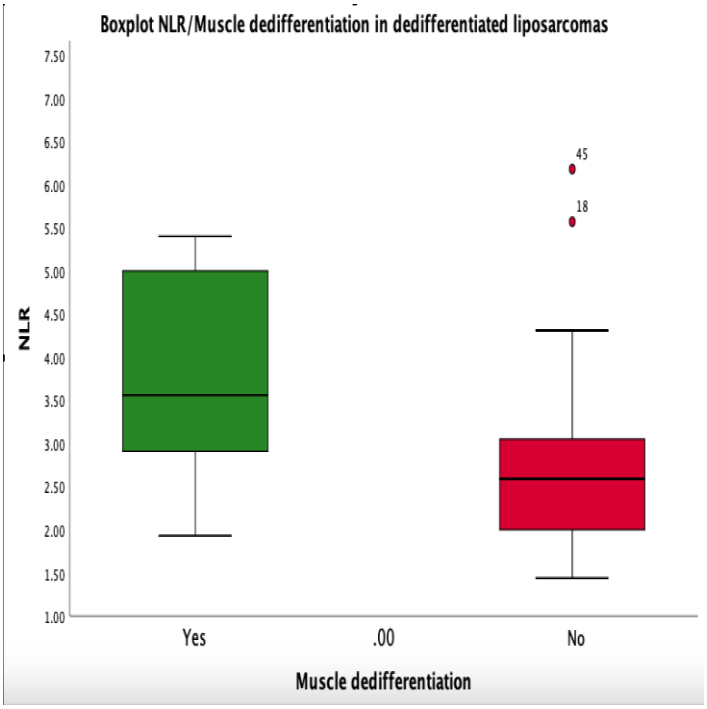
Objective: To investigate the potential association between muscular dedifferentiation in ddLPS and systemic inflammatory profiles using the neutrophil-lymphocyte ratio (NLR) as a surrogate marker for inflammation and as an adverse prognostic factor.

Methods: We conducted a retrospective cohort study utilizing electronic medical records of patients diagnosed with primary retroperitoneal ddLPS between January 2005 and December 2018 at our institution. An immunohistochemical panel consisting of smooth muscle actin, calponin, H-caldesmon, desmin, and myogenin (MyoD) was employed to assess muscular dedifferentiation. Two expert sarcoma pathologists analyzed the samples, determining the presence or absence of these markers. The association of these markers with outcome measures, including DFS and OS, was examined. Additionally, the NLR cut-off point was established using receiver operating characteristic (ROC) curves, and a Pearson correlation analysis was conducted to assess the relationship between myogenic immunohistochemical variables and NLR.

Results: A total of 176 cases were included in the analysis, with 95 being liposarcomas and 50 identified as ddLPS. Among the ddLPS cases, 28 (56%) were male, and the mean age was 54.30 (±13.34) years, with a mean tumor size of 29.28 cm (±11.64 cm). The majority of patients (92%) had locally advanced disease classified as stage IIIB. Muscular dedifferentiation was observed in 30% (n=15) of the ddLPS cases. Although no significant differences were found between muscular dedifferentiation, treatment, and recurrence, distinct patterns emerged in terms of resection status, complications, and reoperations, with higher rates observed in the dedifferentiation group. Furthermore, the group with myogenic dedifferentiation exhibited lower OS (HR 5.08, 95% CI 2.274-11.384, p=0.0001) and DFS (HR 3.79, 95% CI 1.698-8.484, p=0.001). A moderate positive correlation (Pearson correlation: 0.380, p=0.006) was detected between myogenic immunohistochemical variables and NLR, further highlighting the potential association between muscular dedifferentiation and systemic inflammation.

Conclusion: The presence of markers of muscular differentiation is associated with unfavorable prognostic factors, including lower overall survival (OS) and disease-free survival (DFS). Additionally, we found a moderate positive correlation between myogenic immunohistochemical markers and NLR. These findings suggest the potential involvement of a

systemic and possibly local (microenvironmental) inflammatory profile in the genesis and progression of ddLPS. Further studies are needed to elucidate the underlying mechanisms of this association and validate these findings.



(P 332) SURGICAL OUTCOMES OF RETROPERITONEAL LIPOSARCOMA - SINGLE INSTITUTIONAL STUDY N JAPAN -

Aiko Maejima, MD, Ph.D[1]; Motokiyo Komiyama, MD[2]; Satoshi Nara, MD[3]; Konosuke Moritani, MD, Ph.D[4]; Yukinori Yamagata, MD, Ph.D[5]; Yasuo Shinoda, MD, Ph.D[2]; Yoshiyuki Matui, MD, Ph.D[2]; Eisuke Kobayashi, MD[6]; Shintaro Iwata, MD[7]; Akira Kawai, MD, Ph.D[7]; Kan Yonemori, MD, Ph.D[8]; Hiroyuki Fujimoto, MD[2], (1) Department of Medical Oncology/Urology, National Cancer Center Hospital, Tokyo, Japan, Chuuou-ku, Tokyo, Japan, (2) Department of Urology, National Cancer Center Hospital, Tokyo, Japan, Chuou-ku, Tokyo, Japan, (3) Department of Hepatoibiliary and Pancreatic Surgery, National Cancer Center Hospital, Tokyo, Japan, Chuou-ku, Tokyo, Japan, (4) Department of Colorectal Surgery, National Cancer Center Hospital, Tokyo, Japan, Chuou-ku, Tokyo, Japan, (5) Department of Gastoric Surgery, National Cancer Center Hospital, Tokyo, Japan, Chuou-ku, Tokyo, Japan, (6) Department of Musculoskeletal Oncology and Rehabilitation, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (7) National Cancer Center Hospital, Tokyo, Tokyo, Japan, (8) Department of Medical Oncology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan

Objective: Liposarcoma (LPS) is the most common histologic type of retroperitoneal sarcoma, accounting for 50-60% of all cases. Complete tumor resection is the only promising strategy associated with survival benefits. Although the survival outcome of dedifferentiated liposarcoma (DDLPS) is worse comparing to well-differentiated liposarcoma (WDLPS), surgical outcomes of

those are not described precisely.
The aim of this study is to determine whether surgical outcomes differ between DDLPS and WDLPS.

Methods: We retrospectively examined 192 patients with primary retroperitoneal liposarcoma who underwent initial surgery at the National Cancer Center Hospital in Japan between April 2011 and March 2022. Clinicopathological and surgical information were analyzed using the $\chi 2$ and Mann-Whitney tests. Survival analyses with overall survival (OS) and disease-free survival (DFS) were performed using the Kaplan-Meier method. Prognostic factor analysis for survivals was performed using Cox regression analysis.

Results: The histological subtypes included DDLPS (n=121; 61.7%) and WDLPS (n=71; 36.2%). Median follow-up time of all patients was 47 months (range: 3–155 months). Median age at diagnosis was 63 years old (17–82 years old) and 56.6% (n=111) of patients were male. Median operation time was 281 minutes (68–942 minutes) and median blood loss was 658 ml (0–9,524 ml). In 157 cases (80.1%), concomitant organ resection with tumor was performed, with nephrectomy being the most common surgery. DDLPS was characterized by longer operative time (314 vs. 234 minutes, P< 0.001), more blood loss (815 vs. 447 ml, P< 0.001), and more frequent concomitant organ resection (86.8% vs. 73.2%, P=0.003) than WDLPS. Median OS was not reached, the 2-, 5-, and 7-year OS were 89.6%, 77.7%, and 74.6%, respectively. Median DFS was 32 months (95% CI: 5.75-16.24 months), and DDLPS showed worse survival (21.8 months vs. 77.9 months, p< 0.001). The 2-, 5-, and 7-year DFS rates were 62.2%, 32.0%, and 24.3%, respectively. Multivariate analysis for OS demonstrated that a positive margin was at a higher risk of recurrence.

Conclusion: Reviewing the surgical treatment of primary retroperitoneal liposarcoma at our hospital, we found that OS date was good and comparable to the reports of former various authors. This study showed that when LPS was treated by surgery alone, surgery for DDLPS was more invasive that that for WDLPS.

Figure 1-a. Kaplan-Meier curve for death： Overall

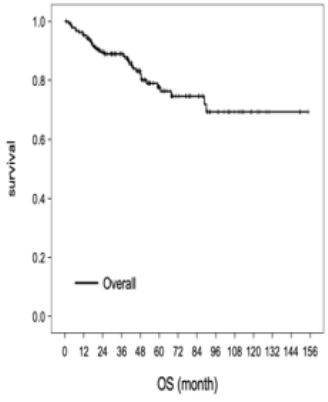


Figure 1-b. Kaplan-Meier curve for death： DDLPS vs. WDLPS

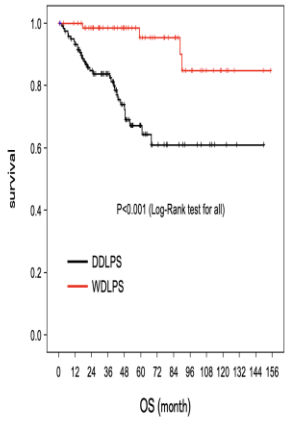


Figure 2-a. Kaplan-Meier curve for relapse： Overall

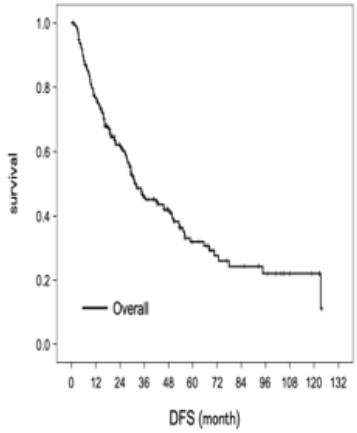
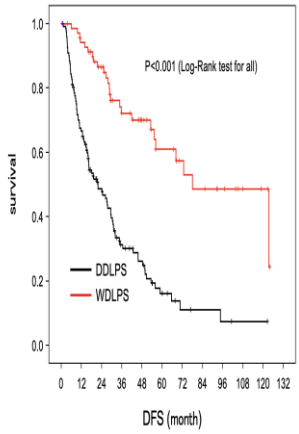


Figure 2-b. Kaplan-Meier curve for relapse： DDLPS vs. WDLPS



(P 333) SYSTEMIC TREATMENT IN INTRA-ABDOMINAL DESMOID-TYPE FIBROMATOSIS: A SINGLE INSTITUTION RETROSPECTIVE CASE SERIES ANALYSIS

Ilaria Pellegrini, MD[1]; Francesca Ligorio, MD[2]; Chiara Colombo, MD[3]; Marco Fiore, MD, FEBS, FACS[4]; Salvatore Provenzano, MD[5]; Carlo Morosi, MD[6]; Ciriaco Buonomenna, MD[7]; Marco Vitellaro, MD[8]; Paola Collini, MD[9]; Alessandro Gronchi, MD, FSSO[10]; Paolo Giovanni G. Casali, MD[11]; Elena Palassini, N/A, MD[12], (1) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (2) Department of Medical Oncology 1, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (3) Department of Surgery, Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (4) Department of Surgery, Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (5) Mesenchymal and rare cancer Medical Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (6) Department of Radiology, Fondazione IRCSS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (7) Department of Radiology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (8) Department of Surgery, Hereditary Digestive Tract Tumors Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (9) Department of Advanced Diagnostics , Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (10) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (11) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (12) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy

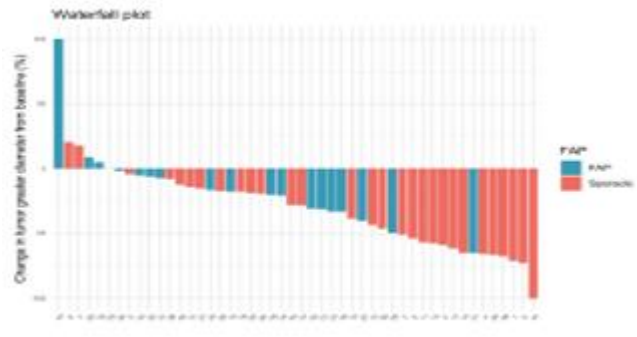
Objective: In the last years the management of desmoid-type fibromatosis (DTF) has changed, privileging a more conservative approach including active surveillance (AS) and limiting surgery. However, extra caution must be used in intra-abdominal DTF because of its critical anatomical site. Moreover, little is known about the role of systemic treatments in this specific subgroup. Herein, we report on the activity of systemic treatments in intra-abdominal DTF.

Methods: Data of patients (pts) with sporadic or FAP-related intra-abdominal DTF treated at our institution from 2007 to 2022 were retrospectively collected and analysed through descriptive statistics. A comparative analysis between FAP-related and sporadic DTF was conducted on patients, tumour characteristics and response to treatment. Tumour response was assessed retrospectively using RECIST criteria and building a waterfall plot of dimensional changes from baseline. PFS was estimated by the Kaplan-Meier method.

Results: 51 pts were included, 20 (39%) with FAP-related and 31 (61%) with sporadic DTF. Males were 27 (53%). The median age was 46 (range 20-77) yrs. Previous treatments were AS in 23 pts (45%), hormonal therapy in 12 (23.5%) and surgery in 16 (31.5%). Low-dose chemotherapy (ld-CT) was used in 49 pts (83%), 2 in second line, anthracyclines in 6 (10%), 4 in second line, and pazopanib in 4 (7%), 2 in second line. Reasons to start treatment included any radiological progression, symptoms and site at high risk of complications. In particular, symptoms were present in 61%, 100% and 50% of pts treated with ld-CT, anthracyclines and pazopanib, respectively. According to RECIST we observed: 1 CR (2%), 24 PR (50%), 21 SD (44%) of which 18 minor responses, including 2 after progression on anthracycline and pazopanib (fig. 1) and 2 PD (4%) with ld-CT; 2 PR (33%), both after progression on ld-CT, 3 SD (50%), including 2 after progression on ld-CT, with remarkable symptoms improvement and 1 (17%) PD with anthracycline; 1 PR (33%), 2 SD (67%), including 1 after progression on ld-CT with symptomatic benefit, with pazopanib. Symptoms improved in 80%, 66% and 50% of pts

treated with ld-CT, anthracyclines and pazopanib, respectively. The median time to response was 11.5 (range 2-29), 6 (range 2-10), and 12 (range 3-14) mth with ld-CT, anthracyclines, and pazopanib, respectively. When considering FAP-related vs sporadic DTF, in the former we saw: 1) lower median age (41 vs 49 yrs; M 43 vs 59 yrs, F 41 vs 43 yrs); 2) more frequent multifocal presentation (50% vs 35%), 3) less AS (40% vs 48%) and 4) lower RECIST response to all therapies (37% vs 59%). Overall, 1 death due to concomitant lymphoma was seen. At a median follow-up (FU) of 33 mth, median PFS was 157 mth in the ld-CT group, with 8 PD; at a median FU of 25 mth (9-73 mth), in the anthracycline group, 1 PD was seen after 2 mth; and in the pazopanib group, with a median FU of 11.5 mth (range, 4-20 mth), we observed 1 PD after 17 mth.

Conclusion: Our analysis (the largest, to our knowledge) reflects our tendency in favour of a more conservative approach even in intra-abdominal DTF. The activity and efficacy of low-dose CT were confirmed, both in FAP-related and sporadic DTF. Anthracyclines may be successfully offered in case of progression and when a fast clinical response is required. New agents may obviously add to them.



(P 334) CHARACTERISTICS AND OUTCOMES FOR PATIENTS IN THE UNITED STATES WITH DEDIFFERENTIATED LIPOSARCOMA FROM A REAL-WORLD DATA SET

Steven Robinson, MBBS[1]; Alyssa B. Klein, MPH[2]; Stephen Stanhope, PhD[2]; Mark Agulnik, MD[3], (1) Mayo Clinic, Rochester, MN, (2) Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, CT, (3) City of Hope, Department of Medical Oncology & Therapeutics Research, Duarte, CA

Objective: Few epidemiological studies describing patients with dedifferentiated liposarcoma (DDLPS) exist, and real-world outcomes data are limited. This study aimed to characterize patients with metastatic DDLPS and assess effectiveness outcomes among those receiving systemic therapy and next-generation sequencing (NGS) in the United States.

Methods: This observational cohort study used the US-based deidentified Flatiron Health-Foundation Medicine (FH-FMI) Pan-Tumor Clinico-Genomic database, which is linked to approximately 280 cancer centers (~800 sites of care). Key inclusion criteria included adults (aged ≥18 years) diagnosed with chart-confirmed metastatic DDLPS from January 1, 2011, to December 31, 2020, who received NGS testing by FMI and received treatment within 14 days before or at any time after the date of metastatic diagnosis. The study period was January 1,

2011 through December 31, 2021. Patients were followed until death, end of study period, or loss to follow-up, whichever occurred first. The index date was the date of initiation of first-line (1L) treatment. Median overall survival (OS) was the time from the index date to the earliest record of death. Real-world progression-free survival (rwPFS) was the time from the index date to the earliest record of disease progression or death based on each patient's chart. Patients without records of death or progression were censored at the last activity date before the end of the study period.

Results: Of 52 selected patients with DDLPS, 43 were diagnosed in 2016 or later. The mean (standard deviation [SD]) age at initiation of 1L treatment for patients with DDLPS was 63.3 (12.8) years. Patients were more likely to be male (63.5%), White (73.1%), and have an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1 (61.5%; ECOG PS missing/unknown in 30.8% of patients). Initial stage at diagnosis was missing in 55% of patients. MDM2 amplification (amp), defined as >8 copies, was seen in 76.9% of patients; 13.5% had a TP53 mutation. Mean time between metastasis diagnosis and start of treatment was 4.2 (9.5) months (mo) and 6.5 (4.5) mo for patients receiving 1L and second-line (2L) treatment, respectively. Median number of treatments received was 2 (IQR, 1-3). Treatments are listed in Table 1. The most common 1L treatment was doxorubicin (doxo) with olaratumab (23.5%), followed by gemcitabine and cisplatin (15.7%). Doxo in combination with other therapies was seen in 15 patients. In 2L, eribulin was the most common (21.9%), followed by gemcitabine (15.6%). OS and rwPFS were 12.6 mo (95% CI, 8.3-19.8 mo) and 2.5 mo (95% CI, 2.3-4.1 mo), respectively, for all patients (n=49). In patients with MDM2 amp and wild-type TP53 (n=37), they were 12.6 mo (95% CI, 6.8-38.0 mo) and 2.5 mo (95% CI, 2.2-5.2 mo), respectively. In patients with a TP53 mutation and no MDM2 amp (n=5), they were 31.5 mo (13.8 mo-not available [NA]) and 4.4 mo (95% CI, 4.1 mo-NA), respectively. In 15 patients (any MDM2 status) who received 1L doxo monotherapy or in combination, OS was 16.6 mo (95% CI, 12.6-NA) and rwPFS was 3.6 mo (95% CI, 2.2-13.2 mo).

Conclusion: Patient demographics are consistent with a 2012 report on 208 patients from 11 institutions (10 European members of the Connective Tissue Cancers Network of Excellence and Memorial Sloan Kettering Cancer Center [New York, NY]); however, median OS observed is lower than the reported 15.2 months. For those receiving 1L doxo monotherapy or in combination, rwPFS was comparable to this report and to a 2017 report on 51 patients at MD Anderson Cancer Center (Houston, TX). These results may highlight an awareness gap between academic centers and community practices for treating DDLPS. Potential limitations include that the data may have a selection bias from the limited number of centers during the study period, and data are derived largely from community practices in the United States and only include patients who also received NGS.

Table 1

First-line therapies (n)	Second-line therapies (n)	Third-line therapies (n)
Doxorubicin + olaratumab (12)	Eribulin (7)	Trabectedin (4)
Gemcitabine + docetaxel (8)	Gemcitabine (5)	Clinical study drug* (3)
Clinical study drug* (6)	Clinical study drug* (3)	Gemcitabine + docetaxel (2)
Doxorubicin (6)	Doxorubicin + olaratumab (3)	Doxorubicin + olaratumab (2)
Pazopanib (4)	Palbociclib (3)	Eribulin (1)
Palbociclib (3)	Doxorubicin (2)	Ifosfamide (1)
Doxorubicin + dacarbazine (2)	Doxorubicin pegylated liposomal (2)	Ifosfamide + mesna (1)
Eribulin (2)	Trabectedin (2)	Olaratumab (1)
Clinical study drug* + pembrolizumab (1)	Gemcitabine + docetaxel (1)	Palbociclib (1)
Doxorubicin + cyclophosphamide + mesna (1)	Doxorubicin + ifosfamide + mesna (1)	Pembrolizumab (1)
Daratumumab (1)	Doxorubicin + imatinib + olaratumab (1)	
Doxorubicin + ifosfamide + mesna (1)	Nivolumab (1)	
Doxorubicin + ifosfamide + mesna + olaratumab (1)	Pazopanib (1)	
Larotrectinib (1)		
Olaratumab (1)		
Trabectedin (1)		

*Clinical study drug not identified.

(P 335) EXTRACELLULAR VESICLE MDM2 DNA AS A BIOMARKER OF TUMOR PRESENCE AND RECURRENCE IN RETROPERITONEAL LIPOSARCOMA

Valerie P. Grignol, MD[1]; Federica Calore, PhD[2]; Patricia Sarchet, BS[2]; Lucia Casadei, PhD[2]; Fernanda Costas casal de Faria, PhD[2]; Raphael E. Pollock, MD, PhD[1], (1) The James Cancer Hospital / The Ohio State University, Columbus, OH, (2) The Ohio State University Comprehensive Cancer Center, Columbus, OH

Objective: A hallmark of well-differentiated and de-differentiated (WD/DD) retroperitoneal liposarcoma is elevated MDM2 due to genome amplification and recurrence rates of >50% even after complete resection. Surveillance imaging frequently cannot resolve recurrent WD/DD-RLS versus postoperative scarring. Early detection of recurrent lesions, for which biomarkers are lacking, would enable more timely therapeutic interventions. We sought to assess the levels of MDM2-DNA within extracellular vesicles (EVs) isolated from the serum of retroperitoneal liposarcoma (RLS) patients versus healthy donors, as well as within the same patients at the time of surgery versus post-operative surveillance visits to identify whether EV-MDM2 may serve as a potential biomarker for liposarcoma recurrence.

Methods: WD/DD-RLS serum samples (n=42) were collected both at the time of surgery and during several follow-up visits, along with sera from healthy donors (n=14). EVs were isolated, DNA purified and MDM2-DNA number of molecules (NM) was determined through q-PCR. Wilcoxon rank sum test was employed to compare patient versus control groups, while Wilcoxon signed-rank tests were used to compare MDM2-DNA levels at the time of surgery versus post-surgery conditions.

Results: EV-MDM2 DNA levels were significantly higher in WD/DD-RLS than controls {mean: controls NM=31,213 [log2 = 14.60; SD = 1.00], time of surgery NM=1,265,643 [log2 = 16.66; SD = 2.58]; (p= 0.00085)}. EV-MDM2 DNA levels were remarkably decreased in WD/DD-RLS patients after resection {mean time of surgery, NM=1,265,643 [log2 = 16.66; SD = 2.58]; mean post-surgery, NM=63,225 [log2 = 15.23; SD = 1.56] (p=0.00036)}, reaching values comparable to control group (p=0.124). Longitudinal values were available in a subset of patients (n=10). Five patients did not develop recurrences and values remained low during surveillance visits over a follow-up period of 6-48 months. In 5 patients who did develop recurrence EV-MDM2 DNA values increased at the time of recurrence as coincident with imaging detected recurrence and decreased again post- resection.

Conclusion: Serum EV-MDM2 is able to differentiate the presence of tumor in patients with retroperitoneal liposarcoma and may serve as a potential biomarker of early recurrent or post-operatively persistent WD/DD-RLS, a disease currently lacking such determinants.

(P 336) EVOLUTION AND PURSUIT OF EXCELLENCE IN SARCOMA MANAGEMENT IN AN ASIAN TERTIARY SARCOMA SPECIALISED CENTRE

Yi Xin Li, BSc[1]; Joey Tan, MSc, BSc[2]; Qiu Xuan Tan, BSc[2]; Josephine Hendrikson, BSc[1]; Gillian Ng, BS[2]; Ying Liu, PhD, BSc[2]; Clara Chong, PhD, BSc[2]; Chin Jin Seo, MB BCh BAO[3]; Jolene Wong, MD, MSc[3]; Khee Chee Soo, MBBS[3]; Claramae Chia, MBBS[3]; Johnny Ong, MBBS, PhD[3], (1) Duke NUS Medical School, Singapore, N/A, Singapore, (2) Laboratory of Applied Human Genetics, Division of Medical Sciences, National Cancer Centre Singapore,

Singapore, N/A, Singapore, (3) Department of Sarcoma, Peritoneal and Rare Tumours (SPRinT), Division of Surgery and Surgical Oncology, National Cancer Centre Singapore, Singapore, N/A, Singapore

Objective: To broadly characterise all soft tissue and visceral sarcoma resections in Singapore General Hospital (SGH) and National Cancer Centre Singapore (NCCS) across a period of 20 years and delineate the evolution of sarcoma care through a focus on retroperitoneal sarcoma (RPS) resections, comparing postoperative outcomes and overall survival (OS) of RPS patients pre and post establishment of the Department of Sarcoma, Peritoneal and Rare Tumours (SPRinT) in 2018, one of the few dedicated subspecialized units in Southeast Asia that treats sarcoma. We aim to underscore the foundational elements needed for the establishment of analogous units as a reference for other upcoming sarcoma centres.

Methods: All patients who underwent soft tissue or visceral sarcoma resections at SGH and NCCS over a 20-year period from February 2002 to October 2022 were identified and retrospectively reviewed from a prospectively maintained database. Patient, tumour and treatment characteristics were summarised for these patients. For RPS patients, additional parameters such as postoperative outcomes, median survival and surgical subspecialty involvement were summarised.

Results: 899 patients were included in this study. 389 (43.3%) patients had soft tissue sarcomas (STS) and 510 (56.7%) patients had visceral sarcomas with a majority achieving R0/R1 margin status (n= 739, 82.2%). 112 (12.5%) patients had RPS with 77 (8.6%) treated before and 35 (3.9%) treated after the establishment of SPRinT. RPS patients treated pre and post establishment of SPRinT were similar in patient and tumour characteristics. Post-SPRinT RPS patients underwent more multivisceral resections with 30 (85.8%) patients having 2 or more organs resected as compared to 26 (33.8%) in the pre-SPRinT group. Increased surgical subspecialty collaboration was observed in the care of RPS patients post-SPRinT, especially with vascular (51.4% vs 3.9%) and urology teams (28.6% vs 15.6%). For postoperative outcomes, 30-day mortality and need for reoperation were low in both groups, however the proportion of patients with more severe postop complications (Clavien-dindo score ≥3) was greater for pre-SPRinT as compared to post-SPRinT RPS patients (28.6% vs 17.1%). Kaplan-Meier analysis revealed no significant difference in 2-year OS between both groups. Median OS was not reached for majority of the patients and a longer follow-up is needed to delineate between the groups.

Conclusion: SPRinT is a dedicated subspecliaized unit that was created to treat sarcoma from a multidisciplinary angle in order to provide the best outcomes for our patients. The evolution of sarcoma management across 20 years in SGH and NCCS as delineated through the specific example of RPS care pre and post the establishment of SPRinT demonstrates the imperative for all sarcoma patients to be referred to and be managed in a centralised, dedicated high volume centre with a specialised surgical unit and multidisciplinary tumour board (MTB) in order to achieve optimal postoperative outcomes, in accordance with recommendations from international guidelines.

(P 337) CLINICAL OUTCOME OF THE PATIENTS WITH HIGH GRADE UTERINE SARCOMA: A SINGLE CENTRE REAL WORLD EXPERIENCE

Mahabuba Binta Hossain, M.B.B.S, MSc (Translational Oncology)[1]; Alexander Lee, Consultant[2]; Ann Buchan, Sarcoma Specialist Nurse[2], (1) The Christie NHS Foundation Trust, Bolton, England, United Kingdom, (2) The Christie NHS Foundation Trust, Manchester, England, United Kingdom

Objective: Uterine sarcoma is a rare tumour with unclear aetiology that originates from the connective tissue or smooth muscle of uterus and accounts for around 3-7 % among all uterine malignancies (Bužinskienė D, 2018). The diagnosis of uterine sarcomas is challenging due to non-specific presentation and limited imaging modalities. The mainstream of management and diagnostics remains pelvic surgery, however despite radical treatment, uterine sarcomas are associated with poor prognosis with early risk of local recurrence or distant metastasis and approximately 40 % of 5-year survival rate. (Song Z, 2020) (Hinchcliff E, 2022) (Huss A, 2022) (Kyriazoglou A, 2018). We have evaluated the outcomes of patients with high or intermediate grade uterine sarcoma post pelvic surgery, who was on surveillance between sarcoma and gynaecology team at a tertiary centre in Manchester, United Kingdom.

Methods: All patients who attended for follow-up post pelvic surgery in the medical oncology sarcoma clinic between January 2016 to December 2020 were retrospectively identified from the hospital database. Patients' follow-up regime was alternative gynae and sarcoma surveillance 3 monthly till year 2 and then 6 monthly till year 5. Clinical records have been used to obtain histology, follow-up adherence and recurrence. The patients with low grade soft tissue sarcoma and FIGO stage IV at initial presentation has been excluded from the study. The cut off for data collection was 30th March 2023.

Results: A total of 45 patients were identified with high grade or intermediate grade uterine sarcoma consisting of 29 uterine leiomyosarcoma, 10 uterine adenosarcoma, 3 epithelioid stromal sarcoma, and 3 undifferentiated sarcoma. Out of 45 patients who were on surveillance between gynae and sarcoma team, 29 patients have developed either local recurrence and or metastasis to secondary sites. Majority of the patients developed either metastasis in lung and or recurrence in pelvic, peritoneum.
More than half of the patients (64%) were progressed during this 5-year surveillance period, with as early as immediately a month post-surgery to 54 months with a median time to progression of 10 months. Among the 29 patients, 20 patients (68%) developed recurrence or metastasis within the 1st year of follow-up, 2 patients in second year, and 2, 4, 1 patient in year three, four and five respectively. Out of 29 patients, 25 patients (86%) died during these follow-up period. 17 out of the 25 patients (68 %) dying within the 1st year of follow-up and 3 patients died within a month since recurrence. The median overall survival period was 7 months since recurrence and the overall 5-year survival rate is 44 %.

Conclusion: We found that the clinical outcomes in this cohort of patients with high grade uterine sarcoma were similar to previous studies. Despite radical treatment and active surveillance, the overall prognosis, and survivability are still poor.

Table 1: Site of recurrence / secondary metastasis

Area of Recurrence / Metastasis	No of patients
Lung	18
Pelvic	12
Peritoneum / Omental	7
Bone	4
Abdominal wall	2
Brain	1
Cardiac	1

Image 1: High grade uterine sarcoma recurrence / metastasis sites

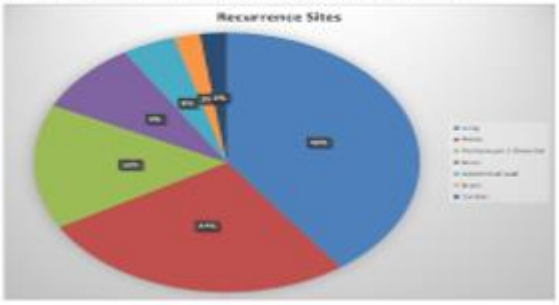
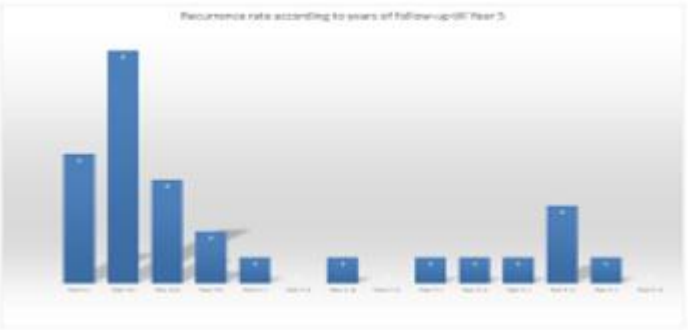


Image 2: Recurrence rate according to years of follow-up till Year 5



(P 338) PREOPERATIVE RADIATION THERAPY IS NOT ASSOCIATED WITH ADVERSE POSTOPERATIVE OUTCOMES IN PATIENTS WITH RETROPERITONEAL SARCOMA

Luc M. Berclaz, MD[1]; Saveli I. Goldberg, PhD[2]; Sonia Cohen, MD, PhD[2]; Edwin Choy, MD, PhD[3]; Gregory M. Cote, MD, PhD[3]; Shannon MacDonald, MD[2]; Thomas F. Delaney, MD[2]; Yen-Lin Chen, MD[2]; John T. Mullen, MD[2], (1) Department of Internal Medicine III, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (2) Massachusetts General Hospital, Boston, MA, (3) Mass General Cancer Center, Boston, MA

Objective: The role of preoperative radiation therapy (preop RT) in the management of retroperitoneal sarcomas (RPS) remains controversial. Due to the radical nature of surgery for RPS, short-term postoperative complication rates are relatively high, and the potential for worse postoperative outcomes with preoperative RT remains a legitimate concern. While median

preoperative radiation doses typically range between 45 Gray (Gy) and 54 Gy in most studies on perioperative outcomes in RPS, protocols at our institution include high preoperative radiation doses up to 63 Gy to the high-risk margin. We sought to examine the 30-day morbidity and 90-day mortality rates associated with high doses of preoperative (+/- intraoperative) RT in patients undergoing curative-intent RPS resection at our institution.

Methods: A total of 99 patients undergoing curative-intent surgery for primary RPS of any histology after preop RT from 2003 – 2021 were identified. All patients received preop RT with or without intraoperative radiotherapy (IORT), followed by macroscopically complete (R0/R1) surgical resection. Patients were excluded if they had multifocal or metastatic disease or if they had surgery or RT at another institution. Clinicopathologic, surgical, radiation, and outcomes data were extracted from our prospectively maintained sarcoma database. Post-operative complications were graded according to the Clavien-Dindo classification system. Univariate and multivariate logistic regression analyses were performed to identify factors associated with postoperative morbidity and mortality.

Results: The clinicopathologic characteristics of the study cohort are summarized in Table 1. Median age was 61 years (range, 25 - 85 years), and 50 (50.5%) patients were female. The most common comorbidities were hypertension and hyperlipidemia. The most common histologic subtypes were dedifferentiated liposarcoma (40%), leiomyosarcoma (26%), and well-differentiated liposarcoma (25%). Most (72%) of the tumors were of intermediate or high grade, and most patients had large tumors, with a median size of 13.5 cm. The median preoperative RT dose was 57.4 Gy, and 17 patients received additional intraoperative RT to a median dose of 10 Gy. Preoperative radiation doses increased over the observation period, from a median dose of 50.4 Gy from 2003 - 2012 to 63 Gy from 2013 - 2021. The median number of organs resected was 2. Postoperative complications within 30 days of surgery occurred in 47% of patients, and severe complications (Clavien-Dindo grades ≥ IIIa) were observed in 20% of patients. The 90-day postoperative mortality rate was 2%. Predictors of severe complications on univariate analysis were en bloc vascular resection (p=0.0002), a higher number of resected organs (p=0.0482), and diabetes as a comorbidity (p=0.0404). Interestingly, the rate of severe complications decreased over the study period, from a rate of 26.3% from 2003-2012 compared to 16.1% from 2013-2021, though this difference was not statistically significant (p=0.3029).

Conclusion: The administration of preoperative +/- intraoperative RT to patients with RPS resulted in similar postoperative complication outcomes as reported after surgery alone, even with RT doses as high as 63 Gy. Continuous advances in surgical and radiotherapeutic expertise resulted in lower complication rates over time despite increasing radiation doses.

Table 1. Patient characteristics.

Factor	Strata	N
Total		99
Sex	Male	49
	Female	50
ASA Class	1	1
	2	38
	3	55
	4	4
	Missing	1
Comorbidities	Hypertension	49
	Hyperlipidemia	38
	Cardiac disease	19
	Diabetes	10
Histological subtype	Dedifferentiated Liposarcoma	40
	Leiomyosarcoma	26
	Well differentiated Liposarcoma	25
	Other	8
Grading	Low (G1)	27
	Intermediate (G2)	34
	High (G3)	37
	Missing	1
Location	Right retroperitoneum	58
	Left retroperitoneum	34
	Midline	2
Size	<50mm (T1)	7
	≥50mm (T2-T4)	92
AJCC stage	Stage I	27
	Stage II	37
	Stage III	34
	Missing	1

Table 2. Preoperative and operative treatment data.

Factor	Strata	N
Total		99
Chemotherapy	Yes	19
	No	80
Intraoperative Radiation	Yes	17
	No	82
Preoperative radiation modality	IMRT + IMPT	33
	IMRT	22
	IMPT	21
	VMAT	7
	VMAT + IMPT	6
	Conventional EBRT	5
	EBRT + IMPT	2
	EBRT + IMRT	1
	VMAT + IMRT	1
	Missing	1
Extent of resection	R0/R1	99
Resection margins	Positive (R1)	25
	Negative (R0)	71
	Missing	3
Number of resected organs	0	4
	1	23
	2	18
	3	23
	≥4	32
Resected organs	Kidney	64
	Colon	60
	Adrenal	26
	IVC	18
	Pancreas	12
	Liver	11
	Spleen	10
	Gallbladder	7
	Testicle	4
	Aorta	3
	Ovary / Fallopian tube	2
	Small intestine	3
	Other	19

Other resected structures included the uterus, lung, bladder, diaphragm, sacrum, femoral nerve, and iliac veins. IMRT=Intensity-Modulated Radiotherapy, VMAT=Volumetric Modulated Arc Therapy, IMPT=Intensity-Modulated Proton Therapy, EBRT=External Beam Radiotherapy

Table 3. Postoperative outcomes.

Factor	Strata	N
Total		99
30-day complication rate	Yes	47
	No	52
90-day mortality rate	Yes	2
	No	97
30-day hospital readmission rate	Yes	14
	No	85
Clavien-Dindo Grading of postoperative complications	Grade I	15
	Grade II	45
	Grades IIIa + IIIb	19
	Grades IVa + IVb	9
	Grade V	2
Complications	Postoperative bleeding	14
	Acute kidney injury/failure	12
	Urinary tract infection	11
	Surgical site infection	3
	Deep surgical site infection	3
	Organ space infection	4
	Sepsis	2
	Septic shock	2
	Pneumonia	6
	Stroke	2
	Ileus	6
	Pneumothorax	4
	Atrial fibrillation	4
	Gastric outlet obstruction	3
	Other	14

Table 4. Univariate and multivariate analyses of major (CD ≥IIIa vs. <IIIa) postoperative complications after preoperative RT.

	Univariate			Multivariate		
	OR	**95% CI**	**P value (Wald)**	**OR**	**95% CI**	**P value (Wald)**
Vascular Resection	8.00	(2.86 – 28.16)	**0.0002**	8.76	(1.81 – 42.32)	**0.0069**
Administration of intraop. RT	0.94	(0.90 – 0.99)	**0.0292**	0.93	(0.87 – 1.01)	0.0902
Resected organs	1.45	(1.00 – 2.12)	0.0526	1.36	(0.82 – 2.27)	0.2298
Diabetes	4.11	(1.11 – 15.24)	**0.0344**	4.65	(0.97 – 22.24)	**0.0538**
Tumor size	0.97	(0.91 – 1.03)	0.3387	0.98	(0.894 – 1.07)	0.6858
Chemotherapy	1.42	(0.37 – 5.43)	0.6117	2.68	(0.44 – 16.03)	0.2788
Age at Diagnosis	1.02	(0.98-1.07)	0.3764	1.013	(0.958– 1.070)	0.6573

(P 339) QUALITY OF SURGERY AND OUTCOME FOR GIANT RETROPERITONEAL LIPOSARCOMA TREATED WITHIN SEMI-ABDOMINAL EN BLOC RESECTION USING VASCULAR PRIORITY CENTRAL APPROACH-A RETROSPECTIVE COHORT

Peidang Fan, Postgraduate/Master[1]; Ping Tao, Postgraduate/Master[2]; Zhenyu Wang, Postgraduate/Master[1]; Jiongyuan Wang, Postgraduate/Master[3]; Lijie Ma, Postgraduate/PhD[4]; Weiqi Lu, Postgraduate/Master[3]; Yong Zhang, Postgraduate/PhD[3]; Hanxing Tong, Postgraduate/PhD[3], (1) School of Medicine, Anhui University of Science and Technology, Huainan, China, Shanghai, CA, (2) Department of Laboratory Medicine, Shanghai TCM-Integrated Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, Shanghai, Shanghai, China (People's Republic), (3) Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai, China, Shanghai, CA, (4) Department of Liver Surgery, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, Shanghai, CA

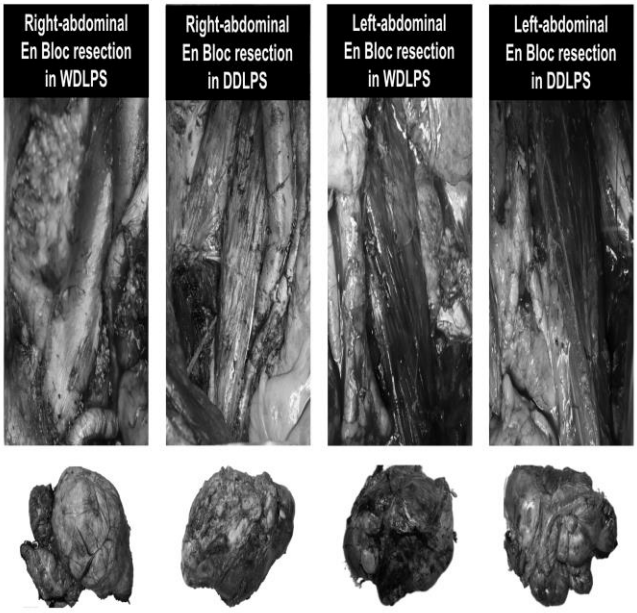
Objective: Retroperitoneal liposarcoma (RPLS) is defined as an aggressive malignant tumor for which adjuvant modalities have not shown increased efficacy compared with surgery, due to the giant tumor burden and characteristics of invasion. The optimal treatment strategy and standardized surgical procedures for giant RPLS are still unknown. In this study, we aimed to evaluate the surgery quality and clinical outcomes of giant RPLS patients with semi-abdominal En Bloc resection using a vascular priority central approach, to determine their potential prognostic and therapeutic utility.

Methods: Retrospective medical record review of a cohort of 132 patients who presented to the Zhongshan Hospital, Fudan University during the period from March 2010 to September 2021. Median follow-up was 37.4 months (IQR, 21.5-71.2 months). Inclusion criteria were confirmed diagnosis of primary or secondary RPLS, with an intermediate or high risk of relapse; no evidence of residual disease after surgery; older than 26 years; and no prior malignancies or concurrent severe/uncontrolled medical conditions. Data were analyzed during the period from September 2022 to May 2023. Demographic and clinical factors were evaluated to assess the clinical course, patterns of recurrence, and survival outcomes for patients with RPLS. Univariate and multivariate Cox proportional hazards models were used to correlate disease-specific overall survival (OS), disease-free survival (DFS), and post-relapse OS with potential prognostic indicators.

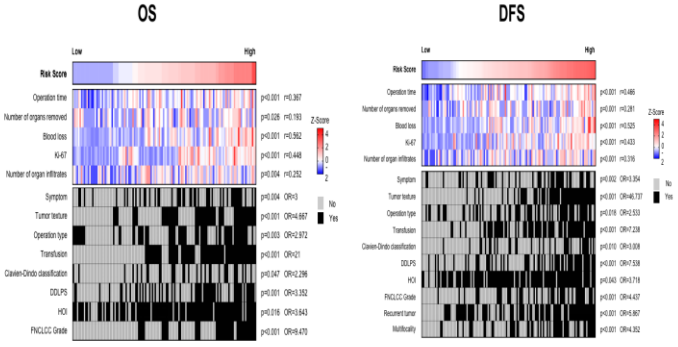
Results: 132 patients with localized RPLS who underwent surgical resection with semi-abdominal En Bloc resection using vascular priority central approach formed the study population, and their clinical features were used for subsequent outcomes analysis (Figure 1).

The median age at diagnosis was 55 years (range, 27-84 years). The majority of patients were male (69 patients [52.3%]). The 5-year disease-specific OS after semi-abdominal En Bloc resection was 62.5%. The median time to OS was 80.5 months (IQR, 21.5-71.2 months), and the median time to post-relapse OS was 24.3 months (IQR, 11.9-36.8 months). The median diameter was 21 cm (IQR, 4.5-47cm) and the median operation time was 5.3 hours (IQR, 2-12 hours). The median number of organs resected was 5 (IQR, 3-8) and the median hospitalization days were 30 days. Severe postoperative adverse events (Clavien-Dindo>3) occurred in 31 patients (23.48%) and none patients died within 30 days. Left-sided RPLS patients receiving semi-abdominal En Bloc resection, longer operation time, more resected organs and blood loss indicated higher operative risk (Figure 2). No impact of postoperative adverse events was found associated with OS, DFS, and post-relapse OS. Significant and independent predictors of OS and DFS were transfusion requirement and FNCLCC grade (Figure 3).

Conclusion: A radical and standardized surgical procedure using a vascular priority central approach for giant RPLS is safe when carried out at a high-flow, experienced specialist sarcoma center. Multivisceral resections should be carefully considered and discussed on an individual basis in RPLS-specific MDT and weighed against anticipated disease biology.



Variables	OR (95% CI)	P value
Gender (female vs. male)	0.518 (0.225-1.192)	0.122
Age（>56 vs. ≤56）	1.289 (0.574-2.891)	0.538
Symptom (yes vs. no)	1.557 (0.681-3.562)	0.294
Nutrition		0.294
0	Reference	
1	1.133 (0.325-3.953)	0.844
2	0.370 (0.102-1.343)	0.13
ASA Score (>2 vs. 1-2)	3.5 (0.669-18.307)	0.138
Operation time (>5.3 vs. ≤5.3)	4.972 (2.113-11.699)	<0.001
Primary tumor (yes vs. no)	1.621 (0.693-3.791)	0.265
Multifocality (yes vs. no)	2.618 (1.151-5.958)	0.022
Diameter（>21.8 vs. ≤21.8）	0.776 (0.346-1.741)	0.538
Tumor texture (hard vs. soft)	2.013 (0.892-4.543)	0.092
Number of resected organs	1.846 (1.301-2.618)	<0.001
Resected organ weighted score	1.980 (1.382-2.837)	<0.001
Operation type (left vs. right)	4.973 (2.021-12.239)	<0.001
Transfusion (yes vs. no)	3.578 (1.462-8.757)	0.005
Blood loss (>800 vs. ≤800）	3.886 (1.623-9.302)	0.002
Histologic subtypes		0.921
WDLPS	Reference	
DDLPS	1.026 (0.448-2.347)	0.952
Others	0.647 (0.071-5.927)	0.7
Ki-67 (>15 vs. ≤15)	2.355 (1.023-5.425)	0.044
FNCLCC Grade (3 vs. 1-2）	0.981 (0.415-2.319)	0.966
HOI (infiltration vs. pushing)	2.599 (0.722-9.357)	0.144
Number of organ infiltrates (>2 vs. ≤2）	3.569 (1.475-8.634)	0.005



(P 340) CENTRAL BOOST RADIATION THERAPY CAN ACHIEVE DURABLE LOCAL RESPONSE IN UNRESECTABLE RETROPERITONEAL SARCOMA

Danielle Burner, na[1]; Peter G. Hendrickson, MD,PhD[2]; Diana M. Cardona, MD, MBA[3]; Dan Blazer, III, MD[1]; James Mullins, CMD[1]; David Kirsch, MD, PhD[4], (1) Duke University, Durham, NC, (2) Duke Univeristy, Durham, NC, (3) Duke University, Duham, NC, (4) Princess Margaret Cancer Centre/University Health Network, Toronto, ON, Canada

Objective: Optimal treatment of retroperitoneal sarcoma (RPS) remains to be defined. Surgical resection is utilized when the patient is fit for surgery and neoadjuvant and/or adjuvant therapy may be helpful for certain histologies in select patients and is being investigated in ongoing clinical trials. For patients with unresectable RPS, treatment may include palliative radiotherapy with standard fraction sizes (1.8 Gy to 2 Gy per daily fraction) and/or systemic chemotherapy. Here, we report the feasibility of using high-dose boost radiation (3 to 4 Gy) to the central part of the tumor in patients with unresectable RPS.

Methods: Five patients with unresectable RPS were treated with radiation therapy using a central boost technique with intensity modulated radiation therapy so that the edge of the tumor received 45 Gy to outer part of the tumor (planning target volume 1 or PTV1) in 1.8 to 2 Gy fractions and the central part of the tumor (PTV2) in 3 Gy to 4 Gy fractions in 5 to 25 fractions. Initial presentation, relevant work-up including imaging and laboratory findings, and delivered radiation treatment is documented and are reported. Locoregional response, the presence of distant disease, overall survival and general adverse events are reported.

Results: Key findings and dosing schedule is summarized in Tables 1 and 2. On average, IMRT doses of 45 Gy – 59 Gy was delivered to the outer part of the tumor (PTV1), while the central part of the tumor (PTV2) received 56 Gy – 75 Gy physical dose, which translates to 61 Gy – 80.2 Gy equivalent dose in 2 Gy fractions (EQD2) in an attempt to improve the response of the RPS to radiotherapy and achieve local control while minimizing the toxicity of treatment. Using this treatment technique, patients experienced mild radiation toxicities overall (CTCAE Grade 1 or 2) such as fatigue, nausea, and diarrhea alternating with constipation. Four of the five patients had clinically stable local disease at the time of their last documented follow up. Unfortunately, despite excellent local response to local radiation therapy, most patients developed metastatic disease (4/5), with the most common sites being lung followed by liver, which ultimately led to early death. In this case series of variable histology, the median survival post radiation therapy was 30 months. At this time, one patient (Case 5) is still alive and on imaging surveillance 36 months after radiotherapy.

Conclusion: Patients with unresectable RPS tolerate IMRT with central tumor boost irradiation well. This approach can delay tumor growth in the majority of patients and allows patients with responsive tumors to live with stable, localized disease for at least 2 to 3 years. To minimize radiation toxicity to the adjacent bowel and other organs while delivering up to 75 Gy to the central part of the tumor, we used sequential, interdigitated, or simultaneous integrated boost techniques. In this small case series of 5 patients, treatment was tolerated well, and excellent local responses were observed regardless of the timing of central boost. Given the high rates of metastatic disease that developed in responding patients, adjuvant systemic therapy may be considered for patients with unresectable RPS treated with aggressive radiation therapy to the central part of the tumor.

Patient ID	Age & Sex	Pathology	RT	Perscription Dose (Gy)	Fraction (Gy)	EQD2 (Gy)	Duration of Tx	Chemotherapy	Site of Metastasis	Stable Disease in RT Field	Survival Post RT
Case 1	62 M	Dedifferentiated Liposarcoma	IMRT	34	2		24 days	N	N/A	Y	30 mos
			Boost	32	4	40					
			Edge Total Dose	54.68							
			Core Total Dose	66		74					
Case 2	19 M	Malignant Peripheral Nerve Sheath Tumor	IMRT	25.2	1.8		48 days	N	Lung	Y	3.5 mos
			Boost	44	4	55					
			Edge Total Dose	58.58							
			Core Total Dose	69.2		80.2					
Case 3	75 M	Low-grade Myxofibrosarcoma	IMRT	36	1.8		43 days	N	Lung	N	24 mos
			Boost	20	4	25					
			Edge Total Dose	48.77							
			Core Total Dose	56		61					
Case 4	68 F	High-grade Leiomyosarcoma	IMRT	34.2	1.8		36 days	Y	Liver, Lung, Lower Extremity	Y	36 mos
			Boost	24	4	30					
			Edge Total Dose	47.57							
			Core Total Dose	58.2		64.2					
Case 5	62 F	Intermediate-grade Leiomyosarcoma	IMRT	45	3		35 days	N	Peritoneal Implants	Y	36 mos (alive)
			Boost	30	1.8	30					
			Edge Total Dose	45							
			Core Total Dose	75		75					

Case 1 CR		Case 2 LA		Case 3 LJ		Case 4 ME		Case 5 WC	
Tx Dates	Comments	Tx Dates	Comments	Tx Dates	Comments	Tx Dates	Comments	Tx Dates	Comments
8/20/14	IMRT	2/16/15	IMRT	8/9/18	Boost Only	6/6/19	Boost Only	2/20/20	SIB
8/21/14	IMRT	2/17/15	IMRT	8/10/18	IMRT	6/7/19	IMRT	2/21/20	SIB
8/22/14	IMRT	2/18/15	IMRT	8/13/18	IMRT	6/10/19	IMRT	2/24/20	SIB
8/25/14	IMRT	2/19/15	IMRT	8/14/18	IMRT	6/11/19	IMRT	2/25/20	SIB
8/26/14	IMRT	2/20/15	IMRT	8/15/18	IMRT	6/12/19	IMRT	2/26/20	SIB
8/27/14	IMRT	2/23/15	IMRT	8/16/18	Boost Only	6/13/19	Boost Only	2/27/20	SIB
8/28/14	IMRT	2/24/15	IMRT	8/17/18	IMRT	6/14/19	IMRT	2/28/20	SIB
8/29/14	IMRT	2/25/15	IMRT	8/20/18	IMRT	6/17/19	IMRT	3/2/20	SIB
9/2/14	IMRT	2/27/15	IMRT	8/21/18	IMRT	6/18/19	IMRT	3/3/20	SIB
9/3/14	IMRT	3/2/15	IMRT	8/22/18	IMRT	6/19/19	IMRT	3/4/20	SIB
9/4/14	IMRT	3/3/15	IMRT	8/23/18	Boost Only	6/20/19	Boost Only	3/5/20	SIB
9/5/14	IMRT	3/4/15	IMRT	8/24/18	IMRT	6/21/19	IMRT	3/6/20	SIB
9/8/14	IMRT	3/5/15	IMRT	8/27/18	IMRT	6/24/19	IMRT	3/9/20	SIB
9/9/14	IMRT	3/6/15	IMRT	9/4/18	IMRT	6/25/19	IMRT	3/10/20	SIB
9/10/14	IMRT	3/9/15	SIB	9/5/18	IMRT	6/26/19	IMRT	3/11/20	SIB
9/11/14	IMRT	3/10/15	SIB	9/6/18	Boost Only	6/27/19	Boost Only	3/12/20	SIB
9/12/14	IMRT	3/11/15	SIB	9/7/18	IMRT	6/28/19	IMRT	3/13/20	SIB
9/15/14	Boost Only	3/12/15	SIB	9/10/18	IMRT	7/1/19	IMRT	3/16/20	SIB
9/16/14	Boost Only	3/13/15	SIB	9/11/18	IMRT	7/2/19	IMRT	3/17/20	SIB
9/18/14	Boost Only	3/17/15	SIB	9/12/18	IMRT	7/3/19	IMRT	3/18/20	SIB
9/19/14	Boost Only	3/18/15	SIB	9/13/18	Boost Only	7/5/19	Boost Only	3/19/20	SIB
9/22/14	Boost Only	3/19/15	SIB	9/17/18	IMRT	7/8/19	IMRT	3/20/20	SIB
9/23/14	Boost Only	3/20/15	SIB	9/18/18	IMRT	7/9/19	IMRT	3/23/20	SIB
9/24/14	Boost Only	3/23/15	SIB	9/19/18	IMRT	7/10/19	IMRT	3/24/20	SIB
9/25/14	Boost Only	3/24/15	SIB	9/20/18	IMRT	7/11/19	Boost Only	3/25/20	SIB

(P 341) A PHASE 1B STUDY TO EVALUATE PREOPERATIVE ERIBULIN IN COMBINATION WITH RADIATION IN RETROPERITONEAL SARCOMA

Limin Zhu, MD PhD[1]; Emile Latour, MS[1]; Byung Park, PhD[1]; Wei Huang, PhD[1]; Skye C. Mayo, MD, MPH[2]; Brett Sheppard, MD[1]; John Vetto, MD[1]; Cristian D. Valenzuela, MD[1]; Robert Eil, MD[1]; Flavio Rocha, MD[1]; Arthur Hung, MD[1]; Christopher W. Ryan, MD[3]; Lara Davis, MD[2], (1) OHSU, Portland, OR, (2) OHSU Knight Cancer Institute, Portland, OR, (3) OHSU Knight Cancer Institute, South Waterfront, Portland, OR

Objective: Management of retroperitoneal liposarcoma (RPLPS) is challenging due to large tumor sizes and complex anatomy. Recurrence rates remain high despite aggressive surgical resections. Preoperative radiation has been evaluated in clinical trials, however its role is not fully established yet. Eribulin is an FDA-approved cytotoxic microtubule inhibitor for the treatment of advanced liposarcoma. The aim of this study was to assess the safety and tolerability of preoperative eribulin in combination with radiation in patients with RPLPS.

Methods: In this open-label, phase 1B, dose-finding study, patients with primary or recurrent resectable retroperitoneal liposarcoma received escalating doses of eribulin and intensity-modulated radiation therapy (IMRT), guided by a modified toxicity probability interval (mTPI) design. Eribulin was administered on day 1 and 8, every 3 weeks for up to 3 cycles. Concurrent radiation began during cycle 1, following day 8 eribulin. The starting dose level of eribulin was 1.1 mg/m2, with dose level +1 escalation dose of 1.4 mg/m2, and the starting dose of IMRT was 50.4 Gy, with dose level –1 de-escalation dose of 39.6 Gy. Surgical resection occurred within 3-10 weeks after completion of radiation therapy. The primary endpoint was to determine the recommended phase 2 dose (RP2D) of concurrent eribulin and radiation. Secondary endpoints included rate of R0 resection, pathologic response, objective response rate (ORR), recurrence free survival (RFS), and overall survival (OS) rates. For exploratory objectives, patients underwent dynamic contrast-enhanced MRI (DCE-MRI) at three different time points during the preoperative period to evaluate for changes in tumor perfusion/permeability and microenvironment during and after chemoradiation. In addition, blood for ctDNA analysis was collected and patient-reported questionnaires (PROMIS) were completed at multiple time-points before and after surgery.

Results: Between June 2018 and January 2023, fifteen patients with resectable retroperitoneal liposarcoma were enrolled in the study. Seven patients were female (46.7%), with ages between 44 and 79 years (median age 62). Both well-differentiated (40%) and de-differentiated (60%) liposarcoma subtypes were enrolled. Thirteen patients were evaluable for dose-determining. Four patients treated at starting dose level had no dose-limiting toxicities (DLTs). Two out of the nine patients treated at dose level 2 had DLTs. One DLT was early resection due to disease progression, and the other one was sudden cardiac death, which was ultimately attributed to cardiac anomaly. There was one related serious adverse event (SAE) of febrile neutropenia. The RP2D was established at eribulin 1.4 mg/m2 and IMRT 50.4 Gy over 28 fractions. Twelve patients underwent surgery after completing preoperative study treatment, with six R0 (50%) and six R1 (50%) resections. The median RFS was 30.4 months (95% CI 12.0-NR), while the 2- and 5-year RFS rates were 71.6% (95% CI 33.7-90.3%) and 47.8% (95% CI 15.3-74.8%) respectively. DCE-MRI, ctDNA and PROMIS data are currently under analysis and will be presented.

Conclusion: A preoperative chemoradiation protocol of eribulin in combination with IMRT showed a manageable safety profile and warrants additional prospective evaluation for treatment of retroperitoneal liposarcoma. (This investigator-initiated trial was supported by Eisai

Inc.; ClinicalTrials.gov number, NCT03361436.)

(P 342) THE EVALUATION AND OUTCOMES OF MESENTERIC AND INTRA-ABDOMINAL DESMOIDS: A TARPSWG STUDY

Tristan Wild, BMSc[1]; Kerianne Boulva, MD[2]; Francesco Barretta, PhD[3]; Kenneth Cardona, MD[4]; Elena Palassini, N/A, MD[5]; Peter Hohenberger, N/A, n/a[6]; Shintaro Iwata, MD[7]; Rebecca Gladdy, MD, PhD[8]; Christina V. Angeles, MD[9]; Neha Goel, MD[10]; Pawel Sobczuk, MD PhD[11]; Samuel Ford, MD, PhD[12]; Greg Sigler, MD[1]; Dan Blazer, III, MD[13]; Winan J. van Houdt, MD, PhD, MS[14]; Stephanie H. Greco, MD[15]; Fabian Johnston, MD, MHS[16]; Vittorio Lorenzo Quagliuolo, MD[17]; Samuel Aguiar, MD[18]; Elisabetta Pennacchioli, MD[19]; Dagmar Adámková Krákorová, MD, PhD[20]; Sameer Rastogi, DM[21]; Chandrajit P. Raut, MD, MSc[22]; Hayden Snow, MD[23]; Palma Dileo, MD[24]; Guy Lahat, MD[25]; Jos A. van der Hage, MD PhD[26]; Attila Kollar, MD[27]; Alessandro Gronchi, MD, FSSO[28]; Carolyn Nessim, MD[29], (1) University of Ottawa, Ottawa, ON, Canada, (2) The Centre hospitalier de l'Université de Montréal (CHUM), Montreal, PQ, Canada, (3) Fondazione IRCCS IStituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (4) Department of Surgery, Winship Cancer Institute, Emory University, Atlanta, GA, (5) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milano, Lombardia, Italy, (6) Sarcoma Unit, Department of Surgery and Mannheim Cancer Center, University Medical Center Mannheim, University of Heidelberg, Mannheim,Germany, Mannheim, Baden-Wurttemberg, Germany, (7) National Cancer Center Hospital, Tokyo, Tokyo, Japan, (8) Mount Sinai Hospital, Sinai Health System, and Princess Margaret Cancer Centre Toronto, ON, Canada Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada University of Toronto, Toronto, ON, Toronto, ON, Canada, (9) Department of Surgery, Rogel Cancer Center, University of Michigan, Ann Arbor, MI, (10) Miami University, Miami, FL, (11) Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (12) Department of Surgery, University Hospitals Birmingham, Birmingham, England, United Kingdom, (13) Duke University, Durham, NC, (14) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (15) Fox Chase Cancer Center, newtown, PA, (16) Johns Hopkins, Baltimore, MD, (17) IRCCS Humanitas Research Hospital, Rozzano, Milan, Lombardia, Italy, (18) A.C. Camargo, São Paulo, Sao Paulo, Brazil, (19) Division of Melanoma, Sarcoma, and Rare Tumor Surgery, European Institute of Oncology, Milan, Lombardia, Italy, (20) Masaryk Memorial Cancer Institute Brno, Czech Republic, Brno, Jihomoravsky kraj, Czech Republic, (21) Department of medical oncology, Dr BRAIRCH, All India Institute of Medical Sciences, New Delhi, Delhi, India, (22) Brigham and Women's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Boston, MA, (23) Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia, (24) University College Hospital, London, England, United Kingdom, (25) Tel Aviv Sourasky Medical Center, Tel Aviv, Tel Aviv, Israel, (26) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (27) Department of Medical Oncology, University Hospital Bern, Inselspital, Switzerland, Bern, Bern, Switzerland, (28) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (29) Department of Surgery, University of Ottawa, Ottawa, ON, Canada

Objective: The natural history of intra-abdominal desmoids is unknown. Literature on desmoids of the extremities or abdominal wall demonstrated that approximately 25-30% regress, 10-15%

rapidly progress, and the majority stay stable. Currently, there are no comparable data on intra-abdominal desmoids and their management. The objective of this study is to characterize their clinical presentation, management, and outcomes.

Methods: Data were collected for eligible patients with intra-abdominal desmoids treated at 38 international sarcoma referral centers from Jan 2008 to Dec 2019. Patients with missing data were excluded. Patients were divided into three cohorts based on their initial management — observation (OBS), medical management (MM), and surgery (SURG) (complete curative vs incomplete palliative resection). Progression-free survival (PFS) was calculated from time of original scan or biopsy (whichever was first) to progression or death for the OBS and MM cohorts. Disease-free survival (DFS) was calculated from date of surgery with complete resection and PFS from the date of incomplete resection to recurrence or progression, respectively, or death for the SURG cohort. For OBS and MM patients, Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 was used to determine status from the initial to final scan in their respective observation period.

Results: We identified 635 patients (50.2% male). Median age at presentation was 43 years (IQR, 32-57) and median follow-up was 48.3 months (25.5-86.8). 101 (15.9%) patients had FAP. The most frequent anatomic site was mesentery (361, 56.9%), followed by retroperitoneum (112, 17.6%), pelvis (92, 14.5%), intra-peritoneal (54, 8.5%), and unknown (16, 2.5%).

Of the 635 patients, 308 (48.5%) underwent upfront complete resection (R0/R1), of which 256 (83.1%) and 52 (16.9%) were elective and emergent, respectively. Incomplete (R2) resection was the index operation in 10.7% (N=68), with 69.1% being elective and 19.1% being emergent (11.8% unknown). Overall, patients who underwent complete resection had a 5-year DFS of 73.4% (CI, 67.6-79.7%), whereas those that had an R2 resection had a 5-year PFS of 83.1% (73.4-94.0%). For surgical patients, on multivariable Cox model, FAP (p=0.004) and non-mesenteric location (p=0.001) were independent adverse prognostic factors for DFS.

Observation was the initial treatment in 18.7% (N=119) of patients while MM was the initial treatment in 22% (N=140) with a 5-year PFS of 66.7% (57.8-77.0%) and 71.4% (63.9-79.8%), respectively.

Of the 119 OBS patients, at a median follow up of 41.8 months, 8% had complete regression (CR), 18% had partial regression (PR), 45% remained stable (SD), and 29% had progressive disease (PD) [0.8%] unknown). Of the 140 initially MM patients, at a median follow-up of 49.1 months 4% had a CR, 33% had a PR, 44% had SD, 19% had PD. Following first line OBS, 20% eventually had surgery, 21% had medical management and 9% had both treatments. Following first line MM, 28% had surgery. Patients who underwent incomplete/R2 resection after non-surgical management (N=17) had a 5-year PFS of 79.9% (61.6-100%). Across all cohorts, at last follow-up, 5.0% of patients died of desmoids and 2.0% of other causes.

Conclusion: Active surveillance in intra-abdominal desmoids is feasible and yields similar percentages of spontaneous disease stabilization and regression as for desmoid tumors at other anatomical sites. Surgery is a valid option when feasible and could be considered as first line in individual patients or second line after PD on a non-operative approach, especially for non-FAP associated mesenteric tumours. Of note overall approximately 10% of patients required an emergent operation and undertaking an incomplete resection was a valid option in selected cases.

Table 1. Demographic, Clinical, and Pathological Characteristics (n=635)

Characteristic	Result
Sex, No. (%)	
Male	319 (50.2)
Female	316 (49.8)
Age, median (IQR), years	43.0 (32.0- 57.0)
Follow-up Period, median (IQR), months	48.3 (25.5-86.8)
Initial Management Cohort, No. (%)	
Observation	119 (18.7)
Observation only	59 (49.6)
Observation then medical management	25 (21.0)
Observation then surgery	24 (20.2)
Observation then medical + surgery	11 (9.2)
Medical management	140 (22.0)
Medical only	101 (72.1)
Medical then surgery	39 (27.9)
Surgical	376 (59.2)
Complete Resection (R0-R1)	308 (81.9)
Elective	256 (83.1)
Emergency	52 (16.9)
Incomplete Resection (R2)	68 (18.1)
Elective	47 (69.1)
Emergency	13 (19.1)
Not indicated	8 (11.8)
Surgery Summary Over Total Course, No. (%)	
Curative intent	362 (57.0)
Palliative intent	87 (13.7)
No indicated	1 (0.2)
No surgery throughout course, No. (% of all patients)	185 (29.1)
FAP, No. (%)	
Yes	101 (15.9)
No	533 (83.9)
Unknown	1 (0.2)
Desmoid Site, No. (%)	
Mesenteric	361 (56.9)
Retroperitoneal	112 (17.6)
Pelvic	92 (14.5)
Intra-peritoneal	54 (8.5)
Not available	16 (2.5)

Table 2. Patient Survival Status at Last Follow-Up by Initial Management

Status	Observation No. (%)	Medical Management No. (%)	Complete Resection No. (%)	Incomplete Resection No.(%)	All Cohorts No.(%)
Alive: No Disease	30 (25.2)	25 (17.9)	213 (69.2)	9 (13.2)	277 (43.6)
Alive: Disease	83 (70.0)	103 (73.6)	68 (22.1)	47 (69.1)	301 (47.4)
Dead: Disease	2 (1.7)	9 (6.4)	12 (3.9)	9 (13.2)	32 (5.0)
Dead: Other Causes	3 (2.5)	2 (1.4)	7 (2.3)	1 (1.5)	13 (2.0)
N/A	1 (0.8)	1 (0.7)	8 (2.6)	2 (2.9)	12 (1.9)
	119	140	308	68	635

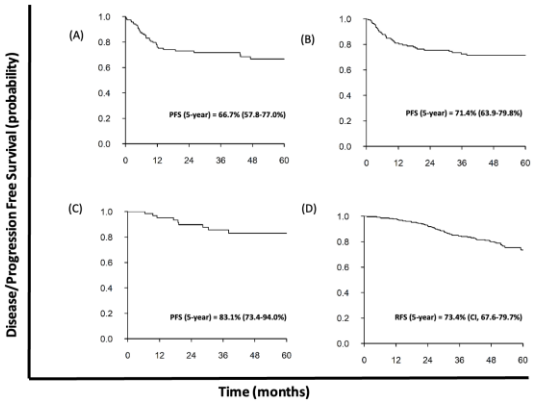


Figure 1(A-E). Progression and Disease Free Survival Curves of Initial Managements
PFS curves were generated for the **(A)** Observational (N=119) and **(B)** Medical (N=139) cohorts from the date of original scan or biopsy (whichever occurred first), and for the **(C)** Incomplete Resection (R2) (N=65) cohort from the date of surgery. 5-year DFS was calculated for the **(D)** Complete Resection (R0-R1) (N=299) cohort from the date of surgery. 5-years PFS/DFS (95% CI) is reported. Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 was used to determine status from the initial to final scan in their respective observation period. Patients with incomplete data were excluded.

(P 343) STEREOTACTIC BODY RADIATION THERAPY (SBRT) IN ADVANCED/RECURRENT RETROPERITONEAL SARCOMA: PRELIMINARY RESULTS

Minsi Zhang, MD, PhD[1]; Yoshiya Yamada, MD[1]; Jacob Shin, MD[1]; Boris Mueller, MD, MPH[1]; Edmund K. Bartlett, MD[2]; Aimee M. Crago, MD, PhD, FACS[2]; Samuel Singer, MD, FACS[2]; Kaled Alektiar, MD, FASTRO[1], (1) Memorial Sloan Kettering, New York, NY, (2) Memorial Sloan Kettering Cancer Center, New York, NY

Objective: The role of radiation in the treatment of retroperitoneal sarcomas (RPS) continues to be controversial. At our institution, we specifically reserve radiation therapy for cases involving borderline-resectable/unresectable primary or recurrent disease. The aim of this study is to evaluate the feasibility and toxicity of stereotactic body radiation therapy (SBRT) in this group of patients.

Methods: Between 1/2017 and 12/2021, 36 patients with nonmetastatic, primary borderline-resectable/unresectable (53%) or recurrent (47%) RPS received SBRT in this retrospective cohort study. Typical expansions from GTV to CTV was 5mm excluding adjacent bowel and other organs at risk, and typical expansions from CTV to PTV was 3mm.

Results: All 36 patients successfully completed the prescribed course of radiation therapy. Tumor size was larger than 10cm in 12 (37%) patients, and histology was dedifferentiated liposarcoma in 23 (64%) patients. The median PTV volume was 338.7 cm3 (range: 21.6-3439.6 cm3), to which a median dose of 30 Gy (range: 25-40 Gy) in 5 fractions was delivered. Nine patients received a simultaneous integrated boost (SIB) to a median volume of 111.7 cm3 (range: 17.2-412.1 cm3), to which a median dose of 40 Gy (range: 30-42Gy) was delivered. The intent of SBRT was preoperative in 64% of patients, and definitive (radiation alone) in 36% of patients. The median follow-up was 14 months. No patient experienced acute grade 3-5 toxicities. Acute grade 1-2 fatigue was observed in 50% of patients, nausea 48%, vomiting 9%, diarrhea 6%, and radiation dermatitis 6%. The overall acute grade 1-2 gastrointestinal toxicity in those who received SIB was 33%, compared to 46% in those who did not (p=0.7). Overall, late complications were observed in 2 patients (6%). One patient who received SIB developed grade 2 persistent nausea and vomiting requiring Reglan. Another patient who did not receive SIB experienced grade 3 gastric bleed. Of the patients treated with a preoperative intent, 14 out of 23 (61%) underwent complete gross resection. Of those 14 patients: 4 patients developed in-field local recurrence (1/8 primary, 3/6 recurrent), and 4 developed out-of-field recurrence (2/8 primary, 2/6 recurrent). The actuarial 2-year local control for those who completed preoperative SBRT followed by complete gross resection was 62%, and that for those who received SBRT alone was 48%.

Conclusion: This study demonstrates that SBRT administered up to 40 Gy in 5 fractions is feasible and well tolerated for patients with primary borderline-resectable/unresectable or recurrent RPS. All patients successfully completed the treatment without any acute grade 3-5 toxicities observed. Only 2 patients (6%) experienced late ≤ grade 3 toxicity. Despite the locally advanced nature of our cohort, in-field local recurrence was observed in only 1/8 patients who had primary borderline/unresectable disease. Further investigations regarding the role of SBRT in RPS, including potential dose escalation trials, are warranted.

WITHDRAWN ~~(P 344) **THE EFFECT OF PRE-OPERATIVE RADIOTHERAPY ON CLINICAL OUTCOME IN RETROPERITONEAL LIPOSARCOMA**~~

(P 345) CLINICIAN SURVEY - CURRENT GLOBAL PRACTICE FOR SARCOMA SURVEILLANCE FOLLOWING RESECTION OF PRIMARY RETROPERITONEAL, ABDOMINAL, AND PELVIC SARCOMA

Danielle J. maes, MD[1]; Samuel Ford, MD, PhD[2]; Olalekan L. Aiyegbusi, MD, PhD[3]; Christel McMullan, PhD[4], (1) University Hospitals Birmingham, Birmingham, England, United Kingdom, (2) Department of Surgery, University Hospitals Birmingham, Birmingham, England, United Kingdom, (3) University of Birmingham, Birmingham, England, United Kingdom, (4) CPROR, University of Birmingham, Birmingham, England, United Kingdom

Objective: Postoperative surveillance following resection of primary retroperitoneal, abdominal, and pelvic sarcoma is standard of care in international high-volume sarcoma centres and has rapidly evolved without an evidence base to become highly intensive and prolonged within most centres. This clinician survey aims to capture a global, contemporary snapshot of international guidelines used to inform sarcoma surveillance following resection of primary retroperitoneal, abdominal, and pelvic sarcoma.

Methods: Between July 2022 and March 2023, a link to an online cross-sectional, self-reported clinician survey consisting of 33 questions to assess the current duration, imaging intervals and imaging modalities used for postoperative surveillance following resection of primary retroperitoneal, abdominal, and pelvic soft tissue sarcoma was sent via individual e-mails to clinicians working at centres which are members of the TransAtlantic Australasian Retroperitoneal Working Group (TARPSWG). In addition, the questions assessed which national or international guidelines form the basis for current practice within the institution. Questions differentiated between surveillance strategies for low-grade and high-grade tumours. The questionnaire was anonymous and distribution and management occurred via the secure JISC Online Survey Platform. Descriptive statistics were calculated for each of the measurement items.

Results: Survey responses were received from 58 different TARPSWG centres. Geographically centres are located in Europe (n=28, 48%), North America (n=20, 34%), Asia (n=5, 9%) South America (n=3, 5%) and Australia (n=2, 4%). The majority of centres use institutional guidelines to guide surveillance intensity (n=43, 74%) and the surveillance imaging modality used (n 39, 67 %). For surveillance intensity and imaging modality, institutional guidelines are partially or entirely based on international guidelines in 81% (n=47) and 78% (n=45) of centres respectively. Influential international guidelines were the Practice Guidelines of the National Comprehensive Cancer Network (NCCN) (n=11, 19%), the Soft tissue and visceral sarcomas ESMO-EURACAN Clinical Practice Guidelines (n=2, 3%), the TARPSWG - Consensus approach on the management of Primary Retroperitoneal Sarcoma in the adult (n=5, 9%) and the UK guidelines for the management of soft tissue sarcomas (n=2, 3%), with 47% (n=27) of centres using a combination of two or more international guidelines to inform their practice. Imaging modalities used for chest surveillance are plain chest radiography (n=7, 12%), non-contrast enhanced CT chest (n=25, 43%), contrast-enhanced CT chest (n=19, 33%), high-resolution CT chest (n=4, 7%), and no chest imaging (n=3, 5%). For abdominal surveillance, the following imaging modalities are used: plain abdominal radiography (n=1, 2%), non-contrast enhanced CT abdomen-pelvis (n=2, 3%), contrast-enhanced CT abdomen-pelvis (n=51, 88%), and MRI abdomen-pelvis (n=4, 7%). Imaging intervals, timing of de-escalation of

imaging intervals and total duration of surveillance for low-grade and high-grade retroperitoneal, abdominal, and pelvic soft tissue sarcoma are shown in Figure 1 and Figure 2, respectively.

Conclusion: This global survey among TARPSWG members demonstrates the heterogeneity in sarcoma surveillance strategies worldwide and emphasises the need for a randomised controlled trial to provide an evidence base for the optimal surveillance schedule following primary resection of retroperitoneal, abdominal, and pelvic soft tissue sarcoma. The results of this survey will be utilised to compare intended versus observed surveillance intensity as part of a retrospective study of surveillance outcomes launched via the TARPSWG platform.

Figure 1: Imaging intervals, timings of de-escalation in imaging frequency and total duration of surveillance for low grade retroperitoneal, abdominal, and pelvic soft tissue sarcoma

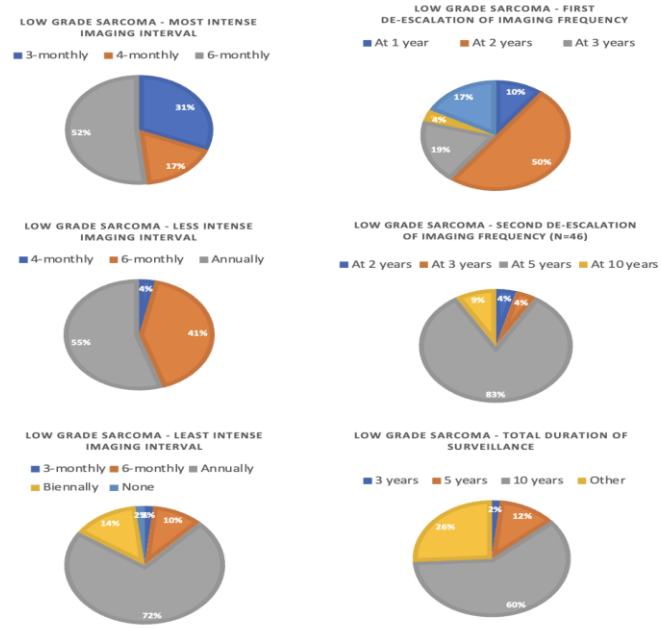
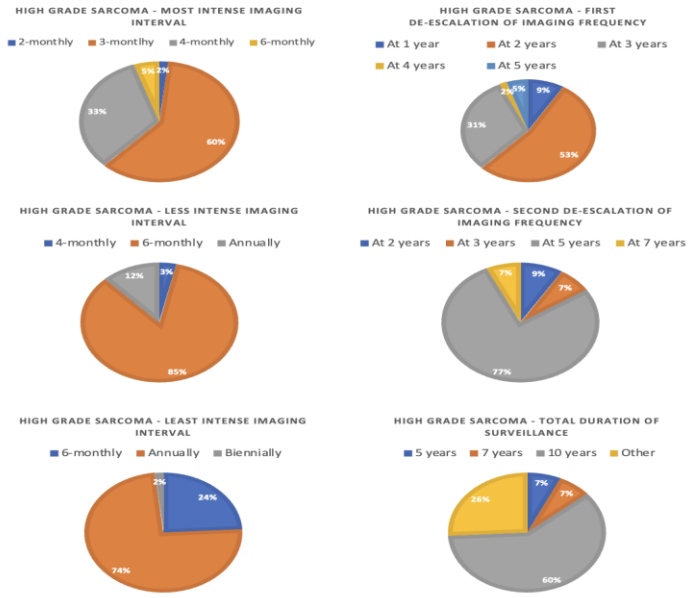


Figure 2: Imaging intervals, timings of de-escalation in imaging frequency and total duration of surveillance for high grade retroperitoneal, abdominal, and pelvic soft tissue sarcoma



(P 346) DETECTION OF GERMLINE VARIANTS IN PATIENTS DIAGNOSED WITH SOFT TISSUE SARCOMA USING EXPANDED

Callisia Clarke, MD[1]; Alexandra C. Istl, MD, MPH[2]; Anai Kothari, MD[2]; John Charlson, MD[3]; Jennifer Geurts, MS, CGC[2], (1) Medical College Of Wisconsin - Surgical Oncology, Milwaukee, WI, (2) Medical College of Wisconsin, Milwaukee, WI, (3) Medical College of Wisconsin, Wauwatosa, WI

Objective: Soft tissue sarcomas (STS) typically present as sporadic tumors with only a small percentage thought to be associated with inherited genetic syndromes. However, several factors point to a strong genetic component as STS disproportionately affects younger patients and STS survivors carry an increased lifetime risk of developing a second primary malignancy. Little is known about the true frequency of germline mutations that may drive STS tumorigenesis. The aim of this study is to characterize the prevalence of clinically actionable germline variants (defined as pathogenic or likely pathogenic) among patients with localized STS.

Methods: This is a retrospective review of a protective cohort of 73 consecutive patients with a diagnosis of STS seen at a single, high-volume sarcoma surgical oncology program and referred for genetic counseling and testing from October 2018 to January 2023. The main outcome measure was identification of clinically actionable germline variants.

Results: Of the 73 patients with STS, 4 (5%) did not have a referral order placed and were not tested despite the recommendation for universal testing. 18 (25%) patients were referred but did not undergo testing, 51 (70%) were seen by a genetic counselor and genetic testing was performed. Of the patients tested 45 (88%) where White/Caucasian, 2 (4%) Black/African American, 1 (2%) Asian, 3 (6%) Hispanic. Six (11.7%) has a personal history of a second primary cancer. Of the 51 patients with genetic testing results, 17 patients (33.3%) had a clinically actionable genetic variant in the following genes: CHEK2 (n=5, 9.8%), BRCA2 (n=1, 1.9%), PMS2 (n=1, 1.9%), MUTYH (n=4 7.8%), WRN (n=1, 1%), APC (n=2, 3.9%), NF1 (n=1, 1.9%), RB1 (n=1, 1.9%), and RAD51 (n=1, 1.9%). Of the 17 patients with a clinically actionable variant, 3 (5.8%) had a personal history of a non-STS cancer (CHEK2 gene; breast cancer), 5 (9.8%) had at least one first-degree relative with a history of any cancers. 15 (29.4%) had variants of unknown significance (VUS) and 8 (15.6%) had no identified variants.

Conclusion: Clinically actionable variants were identified in 33.3% of patients with STS who underwent genetic testing. Family history is not predictive for who will harbor a germline mutation. The detection rate of germline mutations in sarcoma is similar to other cancers (ovarian/pancreas) where we perform genetic evaluation on all and should be considered in all STS patients.

(P 347) INCIDENCE AND TIMING OF RECURRENCE AFTER ADJUVANT PALBOCICLIB IN COMPLETELY RESECTED RETROPERITONEAL LIPOSARCOMA

Samantha M. Ruff, MD[1]; Madelaine Norris, BS[1]; Gabriel Tinoco, MD[2]; David Liebner, MD[3]; Marium Husain, MD[1]; Carlo M. Contreras, MD[4]; Raphael E. Pollock, MD, PhD[4]; Joal D. Beane, MD[5]; Valerie P. Grignol, MD[4], (1) The Ohio State University, Columbus, OH, (2) Ohio State University, Columbus, OH, (3) Ohio State University, James Cancer Hospital and Solove Research Center, Columbus, OH, (4) The James Cancer Hospital / The Ohio State University, Columbus, OH, (5) The James Cancer Hospital / The Ohio State University, Worthington, OH

Objective: Retroperitoneal liposarcomas (RPS) are rare tumors often diagnosed at an advanced stage. While aggressive surgical resection is the cornerstone of treatment for these patients and can be curative, recurrent disease will still occur in 50% of patients at 5 years. As such, research is focused on preventing recurrence or metastatic disease. Our institution has previously demonstrated that Palbociclib, an oral CDK4/6 inhibitor, is well tolerated in the adjuvant setting and may delay the need for additional treatments (e.g. chemotherapy, surgery, radiation) by two years. However, it is unclear which patients derive the greatest oncologic benefit from Palbociclib and how long patients should remain on Palbociclib to prevent or delay recurrence. We sought to evaluate recurrence patterns in patients with high risk retroperitoneal liposarcomas who completed a course of adjuvant Palbociclib without disease recurrence.

Methods: Patients with no evidence of disease (NED) after resection of their RPS and treated

with adjuvant Palbociclib were identified from a prospectively maintained institutional database. Beginning in 2017, high risk patients (e.g. high grade, multifocal disease) were offered adjuvant Palbociclib after complete macroscopic resection (R0/R1) for a period of up to two years. Patients included in this study had NED at the completion of their adjuvant Palbociclib therapy. Time to recurrence was defined as time between completing Palbociclib therapy and recurrence noted on imaging.

Results: Of the thirty-four patients who received adjuvant Palbociclib since 2017, eight met the inclusion criteria of this study. Average age of the cohort was 70y (range: 49-78) and the majority of patients were male (n=6). Most (n=7) of these patients were treated with adjuvant Palbociclib after resection of their primary tumor while one patient received it after resection of a recurrent RPS. Five patients developed a recurrence after therapy cessation. Time to recurrence after completing Palbociclib was within 4 months for most patients (n=4) and 23 months for one patient. The average time on Palbociclib for the patients who recurred was 16.4 months (range: 5-24 months) and for patients who did not recur was 16.3 months (range: 12-23 months). All patients completed at least 12 months of Palbociclib except for one who had to stop therapy after 5 months due to development of bullous pemphigoid. Among the patients with recurrent disease, 3 had de-differentiated RPS and 2 had mixed well and de-differentiated RPS. Among the patients who did not recur, 2 had de-differentiated RPS and 1 had mixed well and de-differentiated RPS. In the cohort of patients who recurred, 3 had FNCLCC grade 3 tumors and 2 had FNCLCC grade 2 tumors. In the cohort of patients currently NED, 2 had FNCLCC grade 2 tumors and 1 had a FNCLCC grade 3 tumor. The three patients without recurrence at the time of this study currently have a disease-free interval of 16, 25, and 33 months since stopping Palbociclib.

Conclusion: Half of the patients in this cohort developed short interval recurrence after completing adjuvant Palbociclib. The optimal duration of adjuvant Palbociclib therapy for patients after RPS resection is not defined nor is how to identify which patients will gain the most oncologic benefit. Studies with larger patient cohorts and genomic analysis of tumors may lend additional insight and is planned for future studies.

	Patients with recurrence after stopping adjuvant Palbociclib n=5 (%)	Patients without recurrence after stopping adjuvant Palbociclib n=3 (%)
Patient Characteristics		
Age (Mean/range in years)	75 (70-78)	61 (49-71)
Sex		
Male	3 (60)	3 (100)
Female	2 (40)	0 (0)
Race		
Caucasian	4 (80)	2 (66)
Unknown	1 (20)	1 (33)
Indication for Surgery		
Resection of primary tumor	4 (80)	3 (100)
Resection of recurrent tumor	1 (20)	0 (0)
Charlson Deyo Score		
0	0 (0)	1 (33)
1	0 (0)	0 (0)
2	0 (0)	1 (33)
3	5 (100)	1 (33)
Tumor Differentiation		
De-differentiated	3 (60)	2 (66)
Mixed type (well and de-differentiated)	2 (40)	1 (33)
FNCLCC Grade		
Grade 1	0 (0)	0 (0)
Grade 2	2 (40)	2 (66)
Grade 3	3 (60)	1 (33)
Time to recurrence after stopping Palbociclib (mean/range in months)	6.6 (1-23)	N/A
Time on Palbociclib (mean/range in months)	16.4 (5-24)	16.3 (12-23)

Table 1: Clinicopathologic characteristics of patients treated with adjuvant Palbociclib after resection of retroperitoneal liposarcoma

(P 348) RESECTION OF RECURRENT RETROPERITONEAL SOFT TISSUE SARCOMAS: ARE INTENSIVE MULTIMODAL TREATMENTS ASSOCIATED WITH INCREASED PERIOPERATIVE MORBIDITY?

Markus Albertsmeier, MD[1]; Mathilda Knoblauch, MD[1]; Lars H. Lindner, MD[2]; Nina-Sophie Schmidt-Hegemann, MD[3]; Jens Werner, MD[1]; Martin K. Angele, MD[1], (1) LMU University Hospital, Munich, Bayern, Germany, (2) Department of Medicine III, LMU University Hospital, LMU Munich, Munich, Bayern, Germany, (3) Department of Radiation Oncology, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany

Objective: Surgical resection is the preferred treatment for local recurrence of retroperitoneal soft tissue sarcomas (RPS). In high-risk tumours, this is often carried out as part of multimodal therapy approaches, which may include radiotherapy, systemic therapy, and – in some centers – regional hyperthermia. Our study aimed to identify clinical, surgical, and therapy-related risk factors for perioperative morbidity following the resection of recurrent RPS within intensive multimodal treatment protocols.

Methods: This study collected clinical characteristics, neoadjuvant therapies, and perioperative complications for patients who underwent surgery for RPS recurrence at our centre between September 2009 and March 2021. The Comprehensive Complication Index (CCI) was calculated for each tumour resection. To account for the specific distribution of CCI values, a zero-one-inflated-beta model for the occurrence of any complication (CCI > 0) and a separate model for 0< CCI< 100 were created and used to analyze the relevance of clinical, surgical, and therapy-related risk factors.

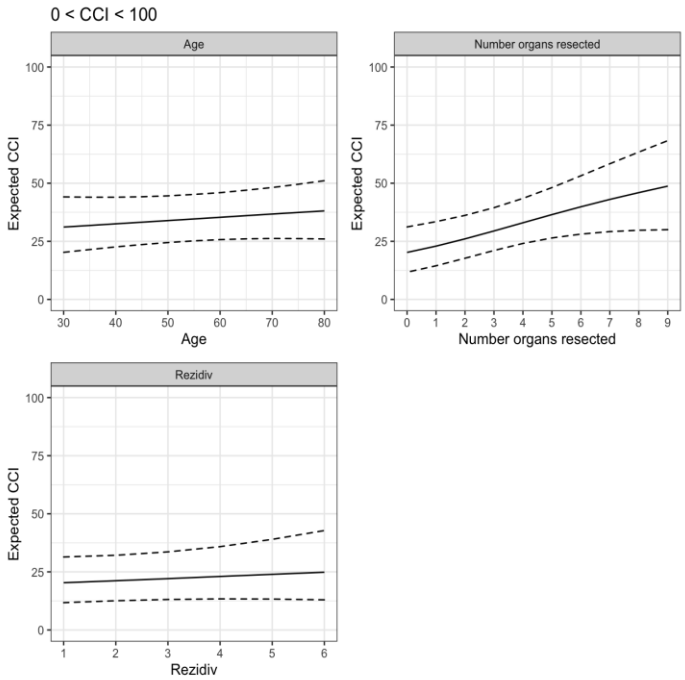
Results: The analysis included 182 patients (91 male, 91 female) who underwent surgery for

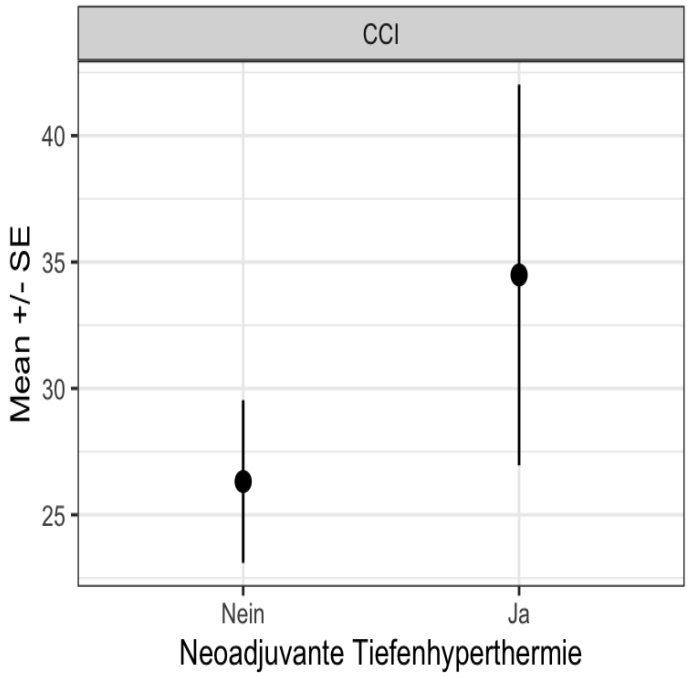
relapsed RPS. The median CCI was 20.9 (range 25%: 0; 75%: 44.9), and it appeared to be higher in patients who underwent neoadjuvant radiotherapy (31.55 (3.05; 42.93) vs 20.9 (0; 45.57) or regional hyperthermia (33.5 (0; 59.2) vs 22.6 (0; 37.08). In a zero-one-inflated-beta model for the occurrence of any complication after recurrent resections (CCI > 0), only the number of resected organs was found to be a significant factor (RR for resection of ≥ 3 organs vs < 3 organs: 1.59 ((1.18, 2.38), p = 0.002). Preoperative therapies, gender, age, transfusion requirements and the number of recurrences had no significant influence on CCI values. This was confirmed in the second model for 0< CCI< 100.

Conclusion: Patients undergoing resections of recurrent RPS experienced postoperative morbidity comparable to primary resections. Operative factors, such as the number of resected organs play a decisive role, while the influence of previous therapies is of secondary importance. Recurrent resections of RPS can be safely performed within the framework of multimodal therapy approaches, but the potential morbidity risk of multivisceral resections should be considered. The CCI is a useful tool to precisely describe perioperative morbidity in sarcoma surgery. Zero-one-inflated beta models can be used to analyze the disproportionate distribution of CCI values.

	Pairwise Comparisons Risk Ratio (95% CI), p-value				
value	Risk CCI > 0				
Therapy		**CTX**	**RAD**	**CTX and RHT**	**CTX and RHT and RAD**
no therapy N = 80	0.72 (0.59, 0.81)	1 (0.56, 1.37) p = 0.988	1.06 (0.7, 1.41) p = 0.726	1.01 (0.72, 1.29) p = 0.958	1.17 (0.86, 1.48) p = 0.256
CTX N = 5	0.72 (0.38, 0.94)		1.06 (0.65, 2.01) p = 0.818	1 (0.65, 1.87) p = 0.992	1.16 (0.77, 2.17) p = 0.468
RAD N = 7	0.76 (0.49, 0.95)			0.95 (0.64, 1.47) p = 0.786	1.1 (0.74, 1.74) p = 0.606
CTX and RHT N = 12	0.72 (0.5, 0.89)				1.16 (0.8, 1.71) p = 0.382
CTX and RHT and RAD N = 7	0.84 (0.6, 0.97)				
Gender		**male**			
female N = 54	0.77 (0.63, 0.88)	0.94 (0.8, 1.1) p = 0.42			
male N = 57	0.72 (0.58, 0.84)				
Transfusion		**yes**	**Missing**		
no N = 69	0.75 (0.61, 0.86)	1.1 (0.9, 1.37) p = 0.318	0.87 (0.64, 1.07) p = 0.196		
yes N = 19	0.83 (0.67, 0.95)		0.79 (0.55, 1.01) p = 0.058		
Missing N = 23	0.65 (0.45, 0.82)				
Age		**>= 70**			
< 70	0.74 (0.6, 0.85)	1.02 (0.88, 1.19) p = 0.756			
>= 70	0.76 (0.61, 0.87)				
Number of organs resected		**>= 3**			
< 3	0.53 (0.34, 0.71)	1.59 (1.18, 2.38) **p = 0.002**			
>= 3	0.84 (0.69, 0.93)				
Rezidiv		**> 1**			
= 1	0.75 (0.62, 0.85)	0.04 (-0.09, 0.15) p = 0.526			
> 1	0.78 (0.63, 0.89)				

Predictor	Pairwise Comparisons Diff. in Means (95% CI), p-value CCI				
Therapy		**CTX**	**RAD**	**CTX and RHT**	**CTX and RHT and RAD**
no therapy N = 80	34.9 (25, 45.9)	-12.53 (-26.8, 6.67) p = 0.178	-4.77 (-19.51, 13.95) p = 0.578	2.78 (-11.01, 18.42) p = 0.708	14.35 (-3.91, 33.5) p = 0.128
CTX N = 5	22.3 (9, 43)		7.72 (-15.09, 29.79) p = 0.482	15.19 (-7.07, 35.1) p = 0.168	26.61 (1.22, 50) **p = 0.042**
RAD N = 7	30.2 (15.7, 49.6)			7.48 (-13.55, 26.97) p = 0.468	18.93 (-6.45, 42.44) p = 0.14
CTX and RHT N = 12	37.8 (23.9, 54.1)				11.49 (-11.92, 34.31) p = 0.33
CTX and RHT and RAD N = 7	49.6 (29.4, 69.7)				
Gender		**male**			
female N = 54	36.4 (25.7, 48.4)	-2.43 (-10.98, 5.85) p = 0.568			
male N = 57	34 (24.1, 45)				
Transfusion		**yes**	**Missing**		
no N = 69	35.7 (25, 47.1)	4.91 (-6.99, 18.07) p = 0.426	-6.45 (-16.86, 4.6) p = 0.234		
yes N = 19	40.6 (27.9, 55.2)		-11.33 (-25.44, 2.15) p = 0.094		
Missing N = 23	29.1 (18.2, 42.3)				
Age		**>= 70**			
< 70	33.6 (24.1, 44.3)	3.72 (-4.35, 12.05) p = 0.368			
>= 70	37.4 (26.2, 49.6)				
Number of organs resected		**>= 3**			
< 3	23.4 (15, 33.9)	16.59 (4.08, 29.77) **p = 0.008**			
>= 3	40.2 (28.3, 53.4)				
Rezidiv		**> 1**			
= 1	35.2 (25.7, 45.8)	3.5 (-6.45, 14.34) p = 0.496			
> 1	38.9 (26.5, 52.1)				





Sarcoma Biology & 'Omics

(P 349) THE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR-ASSOCIATED PROTEIN (UPARAP) IS AN ATTRACTIVE TARGET FOR THE DEVELOPMENT OF ANTIBODY DRUG CONJUGATES FOR TREATMENT OF MESENCHYMAL MALIGNANCIES

Agnieszka Wozniak, PhD[1]; Pernille Barkholt, PhD[2]; Chao-Chi Wang, PhD student[1]; Che-Jui Lee, PhD[1]; Lore De Cock[3]; Lars Engelholm, PhD[2]; Carmel Lynch, PhD[2]; Dominik Mumberg, PhD[2]; Patrick Schöffski, MD, MPH[4], (1) Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Vlaams-Brabant, Belgium, (2) Adcendo ApS, Copenhagen, Hovedstaden, Denmark, (3) Department of General Medical Oncology, University Hospitals Leuven and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Oost-Vlaanderen, Belgium, (4) Department of General Medical Oncology, University Hospitals Leuven, and Laboratory of Experimental Oncology, KU Leuven, Leuven Cancer Institute, Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium

Objective: Urokinase plasminogen activator receptor-associated protein (uPARAP) is an endocytic receptor expressed at low levels on selected mesenchymal cell types, which internalizes fragments of collagen and transfers them to the lysosome for degradation. Besides its physiological role, uPARAP is considered a critical modulator of the tumor microenvironment. The aim of the study was to explore the molecular epidemiology of uPARAP in sarcoma tissue to validate this receptor as potential target for the development of antibody drug conjugates (ADCs) in this indication.

Methods: uPARAP expression was assessed by immunohistochemistry in soft tissue and bone sarcoma specific tissue microarrays with specimens from more than 400 individual donors. Expression was scored by a dedicated sarcoma pathologist based on staining intensity (score 0-3) and percentage of uPARAP-positive tumor cells. Tumors were grouped into high, low and negative uPARAP expressing subgroups.

Results: uPARAP was found to be strongly over-expressed in a high percentage of cases of common STS, with variations in terms of incidence and level of expression between histological subtypes. High uPARAP expression (intensity score 2 or 3 and >50% positive tumor cells) was found in bone sarcoma (93% of cases), fibrosarcoma (86%), dermatofibrosarcoma protuberans (85%), synovial (82%), pleomorphic lipo- (67%), dedifferentiated lipo- (65%), myxofibro- (59%), leiomyo- (49%), and myxoid / round cell liposarcoma (25%). Work is ongoing in additional sarcoma subtypes.

Conclusion: Based on protein expression uPARAP is an attractive emerging therapeutic target for the development of uPARAP-binding ADCs in a broad range of mesenchymal malignancies. In addition, expression of uPARAP in STS may serve as a potential marker for patient selection in early clinical studies with uPARAP-targeting ADCs.

(P 350) LIVER ANGIOSARCOMA IS ASSOCIATED WITH ARISTOLOCHIC ACID EXPOSURE AND HAS A DISTINCT TELOMERE MAINTENANCE MECHANISM

Tom Wei-Wu W. Chen, MD, PhD[1]; Chia-Lang Hsu, PhD[1]; Yu-Ching Wu, PhD[1]; Da-Liang Ou, PhD[2]; Yung-Yeh Su, MD PhD[3]; Ann-Lii Cheng, MD, PhD[1]; Chiun Hsu, MD, PhD[4], (1) National Taiwan University Hospital, Taipei, Taipei, Taiwan (Republic of China), (2) Graduate Institute of Oncology National Taiwan University College of Medicine, Taipei, Taipei, Taiwan (Republic of China), (3) National Health Research Institute, Tainan, Tainan, Taiwan (Republic of China), (4) National Taiwan University Cancer Center, Taipei, Taipei, Taiwan (Republic of China)

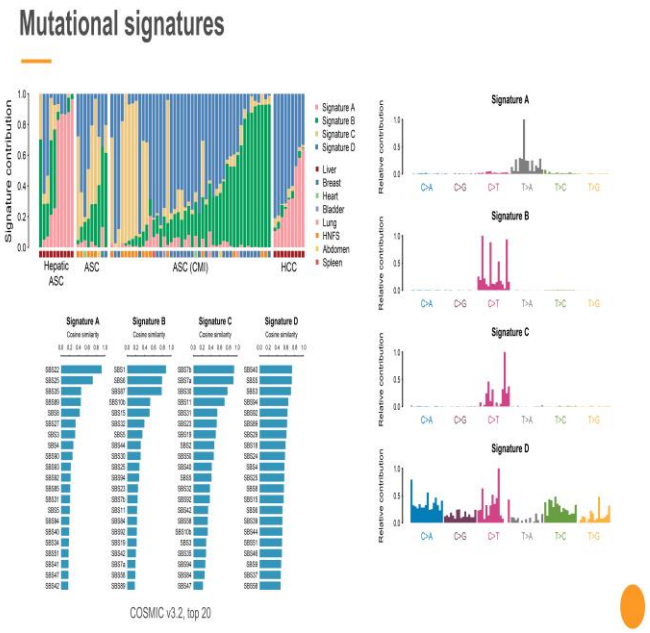
Objective: Liver angiosarcoma (AS) has a grave prognosis and our previous study (Chen TW et al. Cancer 2022) showed that the incidence of liver AS is higher in Asia. We aimed to investigate the etiology of liver AS and its molecular characteristics.

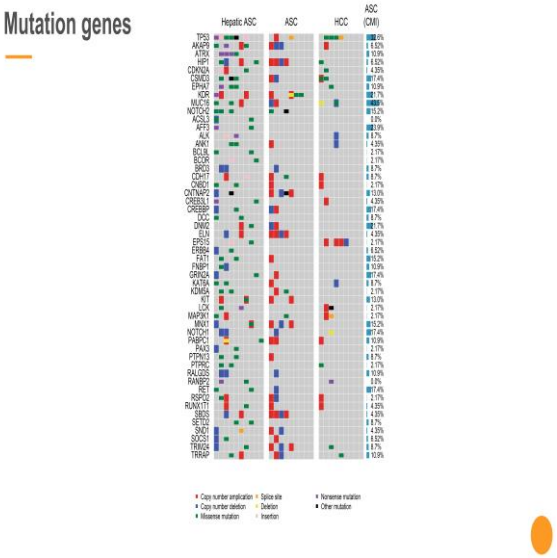
Methods: We performed whole exome (WES) and transcriptome sequencing (RNA-seq) of AS (liver 10, scalp 5, breast 2, trunk 1, heart 1) and hepatocellular carcinoma (HCC, 9) from National Taiwan University Hospital (NTUH) and re-analyzed WES & RNA-seq of AS from the Count Me In Project (CMI, 46). Somatic mutations and copy number alternation were called by Mutect2 and Sequenza from tumor-normal matched WES. Non-negative matrix factorization was used to determine mutational signature profiles and the contributions of each signature to each sample. Telomere length was estimated using TelSeq

Results: We identified four single-base-substitution (SBS) signatures using somatic mutations from 74 (AS/HCC 65/9) WES data. The dominated signature in liver AS had exceptionally high cosine similarity (93.08%) with COSMIC SBS22 (ver 3.2), which is related to aristolochic acid (AA) exposure. The AA-mutation signature contributed significantly (adjusted $p < 0.01$) to the DNA mutations in 70% (7/10), 0% (0/9), 7% (3/46), and 56% (5/9) of liver AS (NTUH), nonliver AS (NTUH), CMI AS, and HCC (NTUH), respectively (Figure 1). TP53 (60% vs 22% vs 32%) and ATRX (40% vs 0% vs 11%) mutations were more common in liver than nonliver AS

(NTUH) or CMI, while KDR mutation rates were similar (30% vs 44% vs 22%) (Figure 2). Among TP53 mutations from liver AS with AA signature, 86% (6/7) showed the typical A:T>T:A mutation caused explicitly by AA-induced DNA damage. In liver vs nonliver AS, the median number of mutations and neoantigen burdens were 6.55 vs 0.98 (p = 0.0076) and 1572 vs 544 (p = 0.095), respectively. Although liver AS and HCC were highly associated with AA-mutation signature, liver AS had significantly longer telomeres in terms of tumor/normal telomere ratio (median 1.27 vs 1.16, p = 0.0009) but a significantly lower telomerase enzyme activity (p = 0.016). We did not find an association between ATRX mutation, or its mRNA expression level, with the extended telomere length in liver AS. Compared to nonliver AS, the activity of the telomere replication molecule BUB1 was significantly higher in liver AS (p = 0.0029).

Conclusion: Our study is the first to connect AA exposure to the etiology of liver AS. The high mutation rate and distinct telomere maintenance mechanism of liver AS provides insights into future treatments.





(P 351) TARGETING ANDROGEN RECEPTOR IN OSTEOSARCOMA CELLS WITH VARYING METASTATIC POTENTIAL

Tanya Heim, M.S.; Murali Kovvur, B.S.; Matthew F. Gong, MD; Ines Lohse, PhD; Kurt R. Weiss, MD, University of Pittsburgh, Pittsburgh, PA

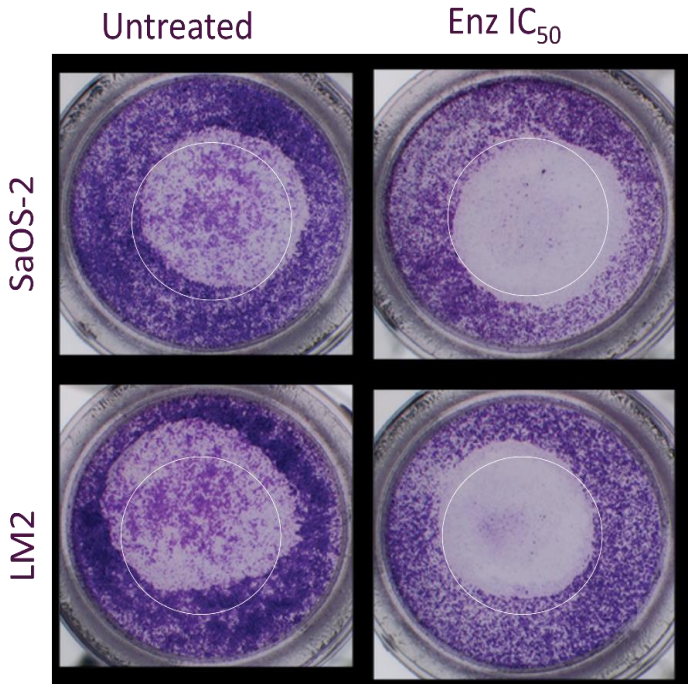
Objective: Lung metastasis (LM) is the primary predictor of mortality in osteosarcoma (OS) patients. Because nearly all cases of metastases (~90%) occur in the lungs, there is a significant need for effective treatment options for this patient population. Previous studies have shown that metastatic OS displays increased expression of aldehyde dehydrogenase 1A1 (ALDH1A1), however, treatment approaches targeting this biomarker alone have not been successful clinically. Androgen Receptor (AR) may be an upstream target of ALDH1A1 based on previous pathway analyses in cohorts of OS patients. Currently FDA-approved for prostate cancer treatment, enzalutamide, is a clinically available AR-inhibitor that can be repurposed for use in OS. In the present study, we aim to validate this hypothesis by evaluating the effect of enzalutamide on the migration of human OS cell lines.

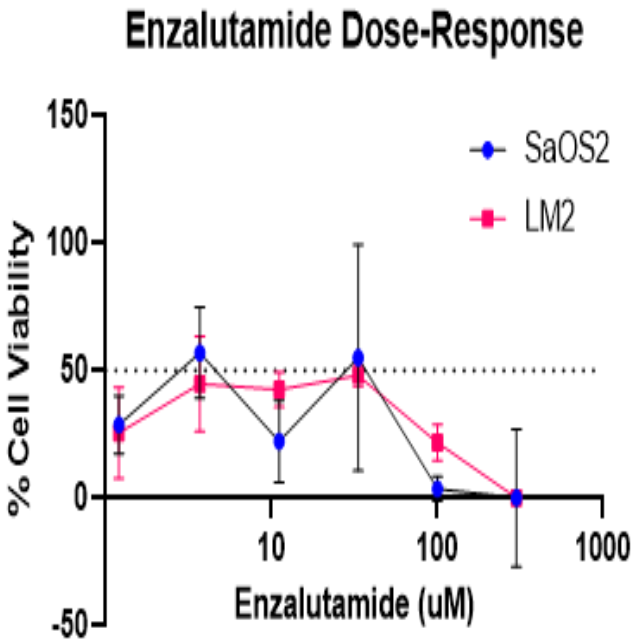
Methods: OS cell lines SaOS-2 (low metastatic potential) and LM2 (high metastatic potential) and dedifferentiated chondrosarcoma cell line HT-1080 (positive control) were cultured. Cells were plated in 60 mm dishes and serum starved for 24 hours during which cells were treated with enzalutamide at their respective IC50 concentration. Next, cells were plated on the transwell insert of a Boyden chamber and incubated for 18 hours, at which point the migrated cells were stained with crystal violet and images were taken. The viability of the OS cell lines when treated with enzalutamide was evaluated using a WST-1 assay (SaOS2) and MTT assay (LM2) per manufacturer's protocol. Briefly, each cell line was plated into a 96-well plate at a density of 25,000 cells. Cells were allowed to adhere overnight. Once adhered, cells were treated with enzalutamide ranging from 300 µM to 1.2 µM or not treated (control). Once incubation with viability assay reagents was complete, the absorbance of each well was measured at 460 nm (SaOS-2) or 570 nm (LM2).

Results: Our preliminary results suggest that treatment with enzalutamide decreased migration

in both OS cell lines, regardless of their metastatic potential. However, the highly metastatic LM2 cells displayed a larger magnitude of sensitivity to enzalutamide (Figure 1). Similar to previous observations, all serum-free (negative controls) displayed significantly lower migration potential than their experimental counterparts. HT-1080 displayed the highest migration potential under normal culture conditions, as expected. Enzalutamide decreased OS cell viability at all concentrations tested. LM2 displayed particular sensitivity to enzalutamide with all tested concentrations yielding less than 50% cell viability. However, SaOS-2 cell viability was greater than 50% when treated with 3.7 µM (mean = 57%) and 33.3 µM (mean = 55%) enzalutamide. SaOS-2 cells displayed more variability to treatment with enzalutamide than did LM2 (Figure 2). Repeat and validation experiments are ongoing.

Conclusion: The presence of LM remains the most important clinical determinant of OS prognosis, nevertheless few treatment options are available for these patients. Recent studies suggest that upregulation of the AR pathway in human LM tissue samples may provide a therapeutic target for patients with LM resistant to conventional chemotherapy. Similar to what has been observed in prostate cancer, AR overexpression may result in aberrant ALDH1A1 expression in OS patients presenting with LM. This opens the possibility of repurposing FDA-approved prostate treatment such as enzalutamide, for the treatment of metastatic OS. Indeed, preliminary results suggest that treatment with enzalutamide has the potential to reduce OS cell migration. While these results are encouraging, additional studies are needed to elucidate the likely complex relationship between AR and ALDH1A1 and their impact on OS metastasis.





(P 352) DETERMINING THE CONTRIBUTIONS OF STAT1 TO THE UPS ANTI-TUMOR MICROENVIRONMENT

Victoria S. Coward[1]; Nalan Gokgoz, PhD[1]; Kim Tsoi, MD, PhD[2]; Jay Wunder, M.Sc, MD[2]; Irene Andrulis, PhD[3], (1) The Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada, (2) University of Toronto, Toronto, ON, Canada, (3) The Lunenfeld-Tanenbaum Research Institute, Sinai Health System University of Toronto, Toronto, ON, Canada

Objective: Sarcoma is a heterogeneous group of cancers with over 70 subtypes and survival rates have remained relatively stagnant in recent decades. We previously found that a subset of Undifferentiated Pleomorphic Sarcoma (UPS) cases harboured tumor-infiltrating lymphocytes (TILs), expressed high levels of PD-1 ligand (PD-L1) RNA, and had better clinical outcomes than those with low PD-L1 expression. Through RNA sequencing, a list of genes differentially expressed between UPS cases with high and low PD-L1 expression was determined. Genes in the Th1 pathway were significantly overexpressed within this list. We hypothesize that tumor-related genes from the Th1 pathway may be related to an anti-tumor microenvironment. Our aims are to identify the potential roles of Th1 pathway genes, PD-L1 and STAT1, in both cell-autonomous and non-autonomous behaviours. This will be accomplished by characterizing patient-derived PD-L1 high and low cell lines followed by examining the functional differences in these cell lines both in the presence of interferon-gamma (IFN-γ) pathway activation and in the absence of STAT1. Transcriptomic changes, cytokine profile, proliferation, migration, invasion, and autologous TIL-sensitivity are being assessed.

Methods: Sarcoma specimens from patients undergoing open biopsy or surgical resection have been collected for the downstream isolation of both tumor cell lines and the respective TIL cultures. Cell lines were treated with recombinant IFN-γ and analyzed for proliferation by evaluating DNA content over time. The ability to migrate in the presence of IFN-γ was assessed

by tracking cell migration across a scratch/wound over 24 hours. In addition, IFN-γ treated cells were subjected to RNA-seq. STAT1 expression in cell lines was disrupted with CRISPR-Cas9 or siRNA methodologies before IFN-γ treatment. Isogenic cell lines were assessed for transcriptomic changes in addition to changes in proliferation, and migration after IFN-γ treatment. A multiplexed Luminex assay was used to identify what cytokines are secreted from isogenic UPS cell lines at rest and in the presence of activating IFN-γ. The isogenic cell lines will be co-cultured with autologous or HLA-A,B,C blocked TIL populations to identify if a STAT1-dependent role exists in tumor-TIL interactions.

Results: Cell lines from PD-L1 high and low cases show unique rates of proliferation and migration, and this is altered by IFN-γ in some cell lines. Distinct transcriptional programs related to cytokine secretion are present in PD-L1 high cell lines after IFN-γ treatment and suggest these cells may be a source of anti-tumor cytokines. UPS cell lines have been successfully gene-edited to be deficient in STAT1 protein production, and phenotypic characterizations of the isogenic cell lines are underway.

Conclusion: Cell lines derived from PD-L1 high and low UPS cases demonstrate variable rates of proliferation and migration when untreated, however some lines show IFN-γ-dependent changes in these behaviors. Although not all characteristics are impacted by IFN-γ; the upregulation of specific transcriptional programs related to anti-tumor functions were IFN-γ-dependent. Future experiments will explore if IFN-γ pathway activation or disrupted STAT1 protein production can contribute to different cytokine profiles in cell lines, which could suggest tumor-driven microenvironment conditions. By assessing the functional consequences of IFN-γ pathway activation and STAT1-disruption, we aim to clarify how STAT1-directed cellular behaviour may be related to the improved anti-tumor microenvironment of the UPS PD-L1 high group.

(P 353) UPDATED EFFICACY AND SAFETY OF LAROTRECTINIB IN ADULT PATIENTS WITH TRK FUSION SARCOMAS

Ray McDermott, MB, BCh, BAO, PhD, MBA, MRCP[1]; Shivaani Kummar, MD, FACP[2]; David S. Hong, MD[3]; Daniel S. W. Tan, MD[4]; Ricarda Norenberg, MSc[5]; Domnita-Ileana Burcoveanu, MD, MSc[6]; Chiara E. Mussi, MD[7]; Alexander Drilon, MD[8]; Lin Shen, MD[9], (1) St. Vincent's University Hospital and University College Dublin, Dublin, Dublin, Ireland, (2) Oregon Health & Science University, Portland, OR, (3) The University of Texas MD Anderson Cancer Center, Houston, TX, (4) Division of Medical Oncology, National Cancer Centre Singapore and Duke-NUS Medical School, Singapore, N/A, Singapore, (5) Chrestos Concept GmbH & Co. KG, Essen, Rheinland-Pfalz, Germany, (6) Bayer HealthCare Pharmaceuticals, Inc., Basel, Basel-Stadt, Switzerland, (7) Bayer HealthCare Pharmaceuticals, Milan, Lombardia, Italy, (8) Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, New York, NY, (9) Department of Gastrointestinal Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Peking University Cancer Hospital & Institute, Beijing, Beijing, China (People's Republic)

Objective: Neurotrophic tyrosine receptor kinase (NTRK) gene fusions are oncogenic drivers in multiple tumor types, including sarcoma. Larotrectinib is a first-in-class, highly selective, central nervous system-active tropomyosin receptor kinase (TRK) inhibitor, approved for tumor-agnostic use in patients (pts) with TRK fusion cancer, based on a rapid, robust, and durable

objective response rate (ORR) in both adult and pediatric pts with various cancers. Here, we report updated long-term efficacy and safety of larotrectinib in adult pts with TRK fusion sarcomas.

Methods: Adult (≥18 years old) pts with TRK fusion sarcomas treated with larotrectinib were identified from three clinical trials (NCT02122913, NCT02576431, and NCT02637687). Most pts received larotrectinib 100 mg twice daily (BID); one pt received 150 mg BID. Responses were assessed by an independent review committee (IRC) per Response Evaluation Criteria in Solid Tumors v1.1. Data cut off date was July 20, 2022.

Results: At data cut off, 37 adult pts with locally advanced or metastatic disease were eligible for efficacy assessment by IRC. Two (5%) pts had bone sarcomas (one not otherwise specified [NOS] and one chondrosarcoma), five (14%) had gastrointestinal stromal tumors, and 30 (81%) had soft tissue sarcomas (nine NOS; six malignant peripheral nerve sheath tumors; four spindle cell sarcomas; three epithelioid spindle sarcomas; two each of myopericytomas, stromal tumors, and inflammatory myofibroblastic tumors; and one each of fibrosarcoma, dedifferentiated liposarcoma, and synovial sarcoma; Table 1). NTRK gene fusions were identified by next-generation sequencing (NGS) and fluorescence in situ hybridization in 36 and one pt, respectively. Median age was 41 (range 19–70) years. Thirteen (35%), 12 (32%), and 12 (32%) pts had received zero, one, and two or more prior systemic therapies, respectively. The ORR was 57% (95% confidence interval [CI] 39–73): nine (24%) pts had complete responses (CR; including one pathological CR), 12 (32%) had partial responses, nine (24%) had stable disease, and six (16%) had progressive disease; response in one pt (3%) was not evaluable (Table 2). Median time to response was 1.8 months (range 1.0–22.9). Median DoR was 54.7 months (95% CI 17.3–not estimable [NE]); median follow-up (f/u) was 26.7 months. Median PFS was 24.8 months (95% CI 5.5–NE); median f/u was 28.4 months. Median OS was 60.2 months (95% CI 38.5–NE); median f/u was 39.4 months (Table 2). The 48-month rates for DoR, PFS, and OS were 53% (95% CI 27–78), 40% (95% CI 22–59), and 56% (95% CI 35–78), respectively.
Treatment-related adverse events (TRAEs) occurred in 31 (84%) pts, with only one Grade 3 TRAE reported (increased weight). There were two Grade 5 adverse events (both due to disease progression), neither of which were considered related to larotrectinib. There were no discontinuations due to TRAEs.

Conclusion: In this updated analysis, larotrectinib continues to demonstrate a rapid and durable response, extended survival benefit, and a favorable safety profile in adult pts with TRK fusion sarcomas. These results support the wider adoption of NGS panels that include NTRK gene fusions to identify pts who may benefit from treatment.

Table 1. *NTRK* gene fusions by sarcoma type

	Bone sarcomas (n=2)	GISTs (n=5)	STS (n=30)	All sarcomas (N=37)
NTRK1	1	0	18	19
NTRK2	1	0	0	1
NTRK3	0	5	12	17

GIST, gastrointestinal stromal tumor; *NTRK*, neurotrophic tyrosine receptor kinase; STS, soft tissue sarcomas.

Table 2. Response to treatment by sarcoma type

	Bone sarcomas (n=2)	GISTs (n=5)	STS (n=30)	All sarcomas (N=37)
Evaluable patients, n	2	5	30	37
ORR, % (95% CI)	50 (1–99)	80 (28–99)	53 (34–72)	57 (39–73)
Best overall response, n (%)				
Complete response	1 (50)	1 (20)	7 (23)	9 (24)
Partial response	0	3 (60)	9 (30)	12 (32)
Stable disease	1 (50)	1 (20)	7 (23)	9 (24)
Progressive disease	0	0	6 (20)	6 (16)
Not evaluable	0	0	1 (3)	1 (3)
Median DoR, months (95% CI)	9.5 (NE–NE)	17.3 (9.5–NE)	54.7 (23.0–NE)	54.7 (17.3–NE)
Median follow-up for DoR, months	NE	50.4	26.7	26.7
48-month DoR rate, % (95% CI)	0	38 (0–94)	58 (27–88)	53 (27–78)
Median PFS, months (95% CI)	7.4 (3.7–NE)	19.3 (11.1–NE)	24.8 (3.6–NE)	24.8 (5.5–NE)
Median follow-up for PFS, months	NE	52.2	28.4	28.4
48-month PFS rate, % (95% CI)	0	38 (0–94)	42 (22–63)	40 (22–59)
Median OS, months (95% CI)	NE	NE (44.4–NE)	60.2 (38.3–NE)	60.2 (38.5–NE)
Median follow-up for OS, months	37.4	36.6	39.4	39.4
48-month OS rate, % (95% CI)	100 (100–100)	50 (0–100)	55 (33–78)	56 (35–78)

CI, confidence interval; DoR, duration of response; GIST, gastrointestinal stromal tumor; NE, not estimable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; STS, soft tissue sarcomas.

(P 354) ROLE OF APPARENT DIFFUSION COEFFICIENT (ADC) MAP-BASED FIRST AND HIGH-ORDER RADIOMIC FEATURES IN DISCRIMINATING SACRAL CHORDOMAS AND CHONDROSARCOMAS WITH OVERLAPPING CONVENTIONAL IMAGING FEATURES

Behrang Amini, M.D, PhD[1]; Raul F. Valenzuela, M.D[2]; Elvis Duran Sierra, PhD[2]; Mathew Antony, M.D[3]; William A. Murphy, Jr., M.D[4]; Chenglei liu, M.D[5]; William N. Green, M.D[6]; Moreko Griggs, M.D[7]; Wei-Lien Wang, M.D[8], (1) MD Anderson cancer Center, Houston, TX, (2) MD Anderson Cancer Center, Houston, TX, (3) MD Anderson cancer center, Houston, TX, (4) M.D Anderson cancer center, Houston, TX, (5) Shanghai Jiao Tong University School of Medicine, Shanghai, Shanghai, China (People's Republic), (6) Houston Methodist Hospital, Houston, TX, (7) Radiology Partners, Houston, TX, (8) The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: Although most chordomas arise from the midline and chondrosarcoma from the lateral pelvis, approximately 10% of chondrosarcomas occur near the midline, displaying similar imaging features to chondroma, causing a diagnostic challenge. This study aims to determine the diagnostic accuracy of ADC-based first and high-order radiomic features and two novel diffusion indices for differentiating sacral chordomas and chondrosarcomas.

Methods: A retrospective, multi-reader review was performed of 82 pelvic MRIs (42 chordomas and 40 chondrosarcomas) between 12/2014 and 9/2021, split into training (n=69) and validation (n=13) datasets. Lesions were segmented on a single slice on ADC maps derived. 10 ADC metrics were calculated: 8 first-order features (minimum, mean, median, and maximum ADC, and standard deviation, skewness, kurtosis, and entropy) and two novel indices: restriction index (RI, proportion of lesion with restricted diffusion) and facilitation index (FI, proportion of lesion with facilitated diffusion). Inter-reader reliability was assessed using the intraclass correlation coefficient (ICC). Regression trees were used to derive cut points and decision trees. Means and 95% confidence intervals of 107 radiomic features comparing chondrosarcoma vs. chordoma patients were sorted based on mean group differences.

Results: There was good to excellent inter-observer reliability for 8 of the 10 ADC metrics on the training dataset. There was a significant difference ($p < 0.005$) between chordomas and chondrosarcomas for RI, FI, median, mean and skewness using the training dataset. Optimal cut-points for diagnosis of chordoma were RI > 0.015; FI < 0.25; Mean ADC < 1.7 × 10-3 mm2/s; Skewness > 0.177. The optimal decision tree relied on FI. In a secondary analysis, the P-values of 107 radiomic features were grouped based on the feature class. Statistically significant differences ($p < 0.00047$) in chondrosarcoma vs. chordoma were found in 18 of 107 radiomic features, including six first-order and twelve high-order features from four texture classes, including Gray Level Co-occurrence Matrix (GLCM), Gray Level Dependence Matrix (GLDM), Gray Level Size Zone Matrix (GLSZM), and Gray Level Run Length Matrix (GLRLM).

Conclusion: The novel FI index reliably differentiates chordomas (FI < 0.25) from chondrosarcomas. Mean ADC provides a practical alternative to FI for routine clinical use. FI, ADC mean, skewness, and 12 high-order radiomic features can reliably differentiate chordomas from chondrosarcomas.

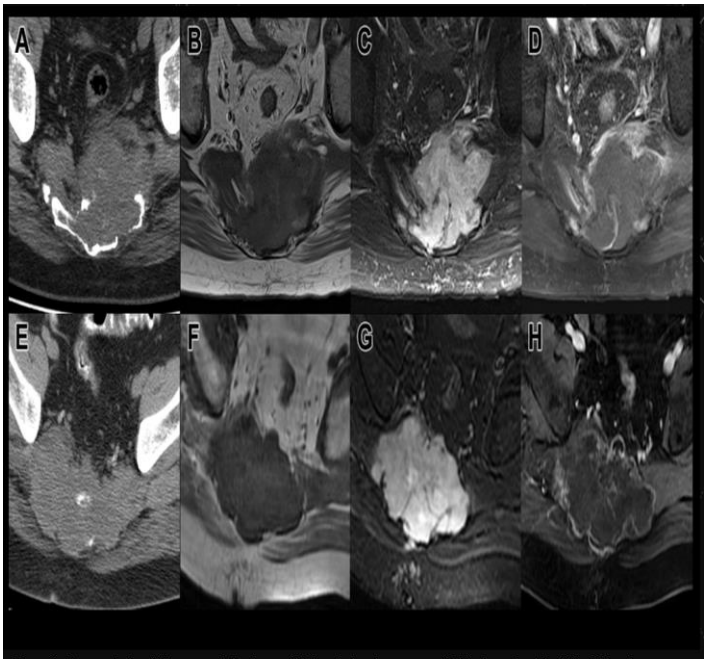


Figure 1. Representative ADC maps of chordomas (A-D) and chondrosarcomas (E-H). Approximately 10% of chondrosarcomas occur near the midline, displaying a similar degree of T2 hyperintensity than chondroma, causing a diagnostic challenge.

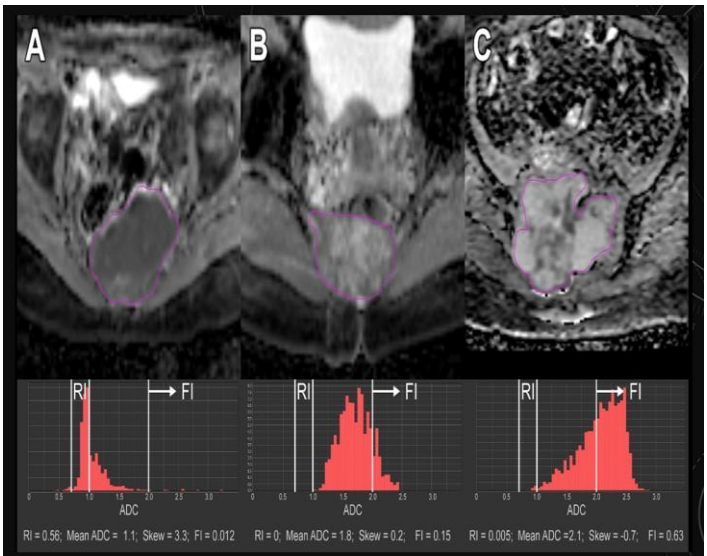


Figure 4. Dedifferentiated chordoma (A) with majority of pixels (RI=0.56) in the range between 0.7-1.0 ×10-3 mm2/s, mean ADC=1.1 ×10-3 mm2/s, positive skewness, and a minimal tail extending beyond 2.0 ×10-3 mm2/s (FI=0.012). Conventional chordoma (B) with no pixels in the range between 0.7-1.0 ×10-3 mm2/s (RI=0.0), relatively high mean ADC of 1.8 ×10-3 mm2/s, minimal positive skewness, and a small number of pixels with ADC > 2.0 ×10-3 mm2/s (FI=0.15). Chondrosarcoma (C) with a very small number of pixels in the range between 0.7-1.0 ×10-3 mm2/s (RI = 0.005), mean ADC=2.1 ×10-3 mm2/s, negative skewness, and most pixels with ADC > 2.0 ×10-3 mm2/s (FI = 0.63).

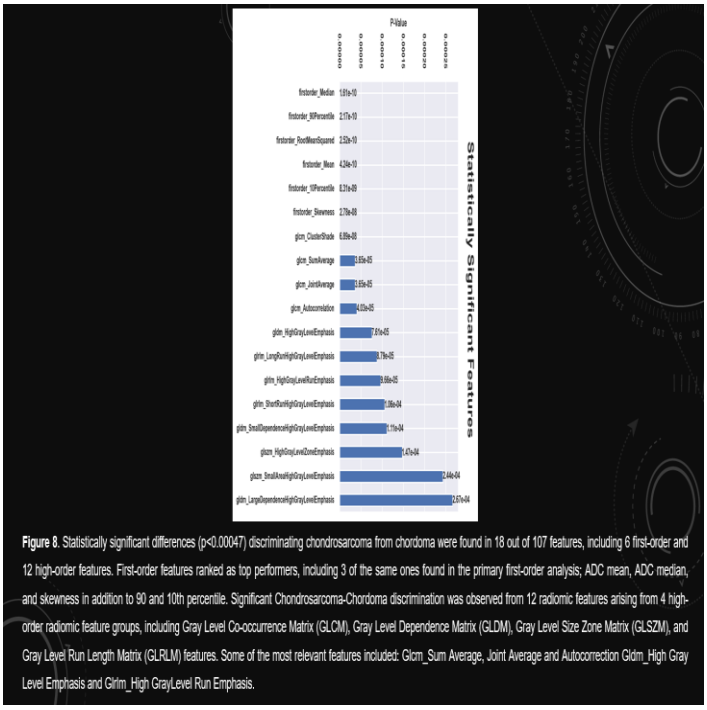


Figure 8. Statistically significant differences (p<0.00047) discriminating chondrosarcoma from chordoma were found in 18 out of 107 features, including 6 first-order and 12 high-order features. First-order features ranked as top performers, including 3 of the same ones found in the primary first-order analysis; ADC mean, ADC median, and skewness in addition to 90 and 10th percentile. Significant Chondrosarcoma-Chordoma discrimination was observed from 12 radiomic features arising from 4 high-order radiomic feature groups, including Gray Level Co-occurrence Matrix (GLCM), Gray Level Dependence Matrix (GLDM), Gray Level Size Zone Matrix (GLSZM), and Gray Level Run Length Matrix (GLRLM) features. Some of the most relevant features included: Glcm_Sum Average, Joint Average and Autocorrection Gldm_High Gray Level Emphasis and Glrlm_High GrayLevel Run Emphasis.

	Training dataset	Validation dataset			
Variable	Cut-point for diagnosis of chordoma	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)	AUC (95% CI)
RI	> 0.015	0.62 (0.26-0.9)	1.0 (0.52-1.0)	0.79 (0.49-94)	0.81 (0.62-.95)
FI	< 0.25	1.0 (0.6-1.0)	1.0 (0.52-1.0)	1.0 (0.73-1.0)	1.0 (1.0-1.0)
Mean ADC	< 1.7 × 10^{-3} mm^2/s	0.75 (0.36-0.96)	1.0 (0.52-1.0)	0.86 (0.56-0.97)	0.88 (0.75-1.0)
Skewness	> 0.177	0.88 (0.47-0.99)	0.83 (0.36-0.99)	0.86 (0.56-0.97)	0.85 (0.65-1.0)

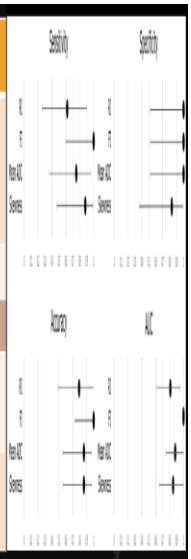


Table 2. The optimal decision tree relied only on FI (sensitivity, specificity, accuracy, and AUC=1). Optimal cut-points for diagnosis of chordoma: RI > 0.015; FI < 0.25; mean ADC < 1.7 x 10-3 mm2/s; skewness > 0.177. Testing cut-points on the validation dataset yielded the highest diagnostic accuracy for FI (sensitivity, specificity, accuracy, and AUC=1).

(P 355) FUSION TRANSCRIPTOME PROFILING DEFINES THE MONOCLONAL NATURE OF MULTIFOCAL EPITHELIOID HEMANGIOMA OF BONE

Ilaria De Benedictis, MSc[1]; Costantino Errani, MD[2]; Alberto Righi, MD[3]; Beatrice Valenti, PhD[1]; Elisa Del Savio, PhD[1]; Davide Baldazzi, PhD[1]; Stefania Benini, M. Sc.[4]; Marta Sbaraglia, MD[5]; Brayan Vega Jimenez, n/a[2]; Daniel Vanel, MD[2]; Davide Maria M. Donati, MD[6]; Angelo Paolo Dei Tos, N/A, MD[7]; Roberta Maestro, PhD[8], (1) Unit of Oncogenetics and Functional Oncogenomics, Centro di Riferimento Oncologico di Aviano IRCCS, Aviano, Italy, Aviano (PN), Friuli-Venezia Giulia, Italy, (2) Istituto Ortopedico Rizzoli, Bologna, Emilia-Romagna, Italy, (3) IRCCS, Istituto Ortopedico Rizzoli, Bologna, Emilia-Romagna, Italy, (4) Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Emilia-Romagna, Italy, (5) University of Padua, Padova, Veneto, Italy, (6) Istituto Ortopedico Rizzoli IOR IRCCS, SC Third Orthopaedic Clinic and Traumatology, Bologna Italy, Bologna, Emilia-Romagna, Italy, (7) Department of Medicine, University of Padua School of Medicine, Padova, Italy, Padova, Veneto, Italy, (8) Unit of Oncogenetics and Functional Oncogenomics, Centro di Riferimento Oncologico di Aviano IRCCS, Aviano, Italy, Aviano, Friuli-Venezia Giulia, Italy

Objective: Epithelioid hemangioma (EH) of bone is a highly controversial entity. Indeed, despite a benign histological appearance, EH of bone may show lymph node involvement and multifocal presentation. Whether multifocal lesions represent independent tumors or metastatic foci is still debated. Although tumor multifocality and lymph node involvement would suggest an aggressive clinical behavior, this is apparently contradicted by some clinical studies. EHs of bone are hallmarked by FOS or FOSB gene rearrangements that may be used in differential diagnosis with EH mimics. In this work we retrospectively analyzed 42 patients treated in a single institution from 1978 to 2021, to describe EH clinical behavior and define the clonal relationships between multifocal lesions, using gene fusion breakpoints as a clonality marker.

Methods: Fusion-targeted analysis was performed using a customized Archer FusionPlex RNA-sequencing panel, supplemented with spike-ins primers for FOS and FOSB. Libraries were run on an Illumina MiSeq sequencing platform. The Archer Analysis bioinformatics suite and in-house-optimized fusion callers (Arriba and Fusion Catcher) were used for fusion transcript identification. Whole transcriptome analysis was performed by RNA-sequencing using the Illumina Stranded Total Ribo-Zero Plus RNA Library Prep kit. Libraries were run on an Illumina Hiseq 1000 platform. PCA and unsupervised hierarchical clustering were performed on the top 5000 genes with the highest variance. RT-PCR and Sanger sequencing were used for orthogonal validations.

Results: Prognosis of our series of EH of bone was excellent. Multifocal synchronous presentation was detected in 17 of 42 patients (40%). Patients were treated with curettage (57%), resection (29%) or biopsy followed by radiotherapy or embolization (14%). Only 5 local recurrences were detected and no death of disease. To clarify whether multifocal EHs represent multicentric disease or metastases, multiple lesions of 3 cases were molecularly profiled. Synchronous EHs from the same patient showed a very similar transcriptional profile and expressed the same fusion transcript (involving FOS or FOSB) with identical gene breakpoints at both RNA and DNA level, demonstrating a common clonal origin.

Conclusion: Our results indicate that EH is a tumor with an excellent prognosis. Molecular analysis of synchronous EH lesions indicates that they are clonally related and therefore represent metastasis of a common original clone. This finding is in apparent contradiction to the benign clinical course of the disease. We propose that tumor multiplicity in EH of bone likely reflects a phenomenon of passive spreading, whereby EH tumor cells, which are facilitated in their capacity to disseminate because of their vascular nature, retain their benign biological nature.

(P 356) BASELINE AND POST-TREATMENT ADVANCED MRI OF EXTREMITIES SOFT-TISSUE NON-MYXOID UNDIFFERENTIATED PLEOMORPHIC SARCOMA.

Raul F. Valenzuela, M.D[1]; Behrang Amini, M.D, PhD[2]; Mathew Antony, M.D[3]; Elvis Duran Sierra, PhD[1]; Colleen Costelloe, M.D[3]; John E. Madewell, M.D[1]; William A. Murphy, Jr., M.D[4], (1) MD Anderson Cancer Center, Houston, TX, (2) MD Anderson cancer Center, Houston, TX, (3) MD Anderson cancer center, Houston, TX, (4) M.D Anderson cancer center, Houston, TX

Objective: To determine the value of advanced MRI with Diffusion-WI (DWI), Contrast-enhanced Susceptibility-Weighted-WI (SWI-CE), and Perfusion-WI (PWI/DCE), in UPS treatment response.

Methods: Retrospective IRB-authorized study included 26 extremity non-myxoid UPS patients with imaging and resection from February 2021-October 2022. Volumetric tumor segmentation from DWI, SWI-CE, and PWI/DCE, were obtained at multiple time points: Baseline (BL), Post-Chemotherapy (PC), and Post-Radiation (PRd).

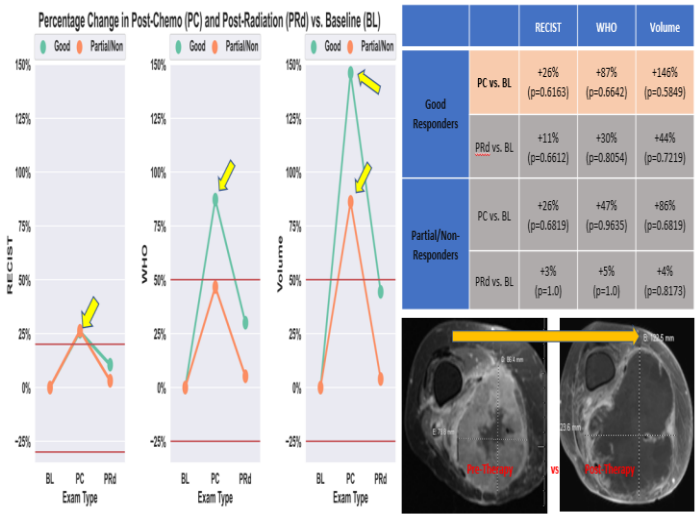
Results: Treatment-induced tumor necrosis (TIN) in surgical specimens separated cases as Good Responders (GR: TIN>90%, 13 patients), Partial-Responders (PR: 89-31%, 8 patients), and Non-Responders (NR: < 30%, 5 patients).

TumorVolume: GR increased by 60% at PC and 90% at PRd. GR baseline ADCmean was 1200, and PR-NR was 1500 x10-6 mm2/sec. At PRd, GR ADC increased by 45% and PR-NR by 30%.

SWI patterns: GR: "complete ring" in 82%. PR: "incomplete ring" in 50%. NR: "globular pattern" in 60%. GR : BL SWImean of 700, decreasing to 100 at PC due to treatment-induced bleeding T2* hypointensity.

PWI/DCE: Type V was the most common time-intensity curve (TIC) at BL. GR: 100% type II TIC at PRd. Post-radiation PWI/DCE patterns: GR: "capsular-like" in 69%. PR: "unipolar" in 87%. NR: "bipolar" in 60%.

Conclusion: RECIST and WHO criteria often cannot separate GR from PR-NR. Post radiation volumetric pseudoprogression, >40% ADC increase, SWI-CE "complete ring", significant decrease in SWImean PWI/DCE Type II TIC and "capsular-like" patterns, correlate with non-myxoid-UPS-with>90% TIN. Suboptimal responders showed incomplete SWI ring TIC III/IV/V, and unipolar or bipolar enhancement. Advanced MRI is a valuable tool for UPS treatment assessment.



		RECIST	WHO	Volume
Good Responders	PC vs. BL	+26% (p=0.6163)	+87% (p=0.6642)	+146% (p=0.5849)
	PRd vs. BL	+11% (p=0.6612)	+30% (p=0.8054)	+44% (p=0.7219)
Partial/Non-Responders	PC vs. BL	+26% (p=0.6819)	+47% (p=0.9635)	+86% (p=0.6819)
	PRd vs. BL	+3% (p=1.0)	+5% (p=1.0)	+4% (p=0.8173)

Figure 1. Stable disease: All good responders and partial/non-responders post radiation tumors are in the range of stable disease by RECIST, WHO and Volume. RECIST, WHO and Volumetric criteria cannot separate good responders from partial/non-responders. Pseudo progression at PC happens on RECIST on all cases, on WHO in good responders and in Volumetric in all cases, and it most significant at the end of systemic therapy. The highest degree of pseudo progression is seen at PC in good responders, with 145% volume increase since baseline. No significance obtained separating good vs partial/non-responders by size metrics.

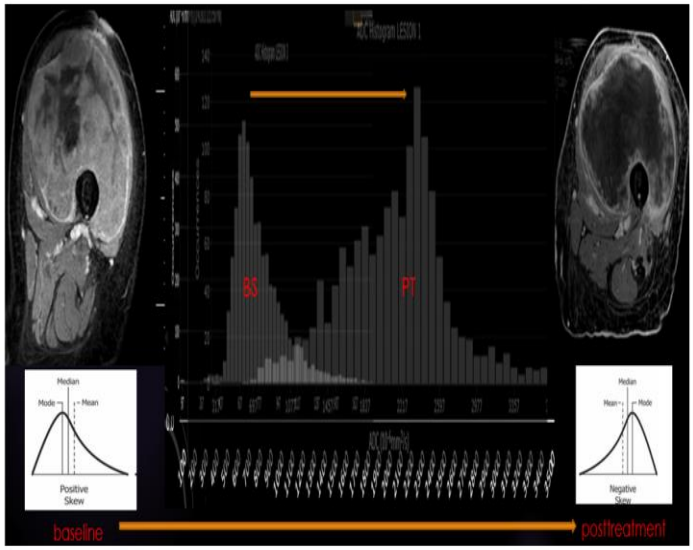


Figure 2. Left: Pretherapeutic (BS) contrast-enhanced MRI of the left thigh with a predominance of solid internal enhancement, associated with a left-sided ADC histogram (positive skewness). Right: Post-therapeutic (PT) contrast-enhanced MRI demonstrating markedly diminished solid enhancement and a right-sided ADC histogram (negative skewness).

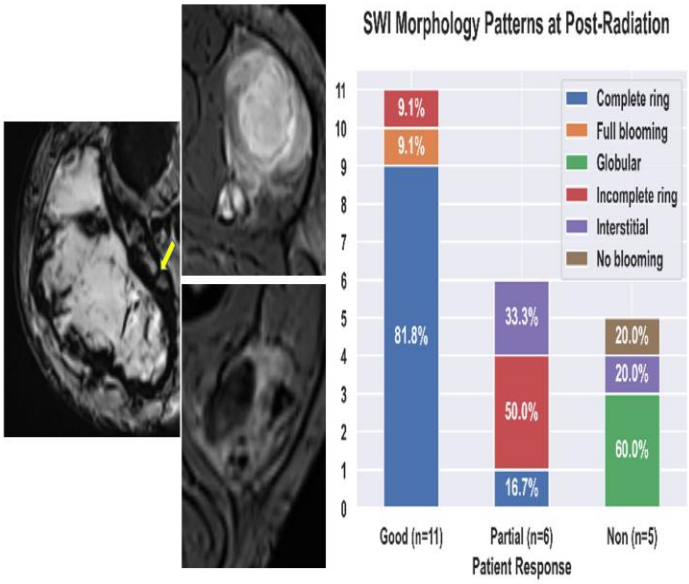


Figure 6. Morphological patterns of SWI at post-radiation. GR displayed "complete ring" in 82%, PR displayed "incomplete ring" in 50%, and NR displayed "globular pattern" in 60%. Complete ring was significantly correlated with GR (p=2.1x10-6), incomplete ring with PR (p=0.1562), and the globular pattern with NR (p=0.1455).

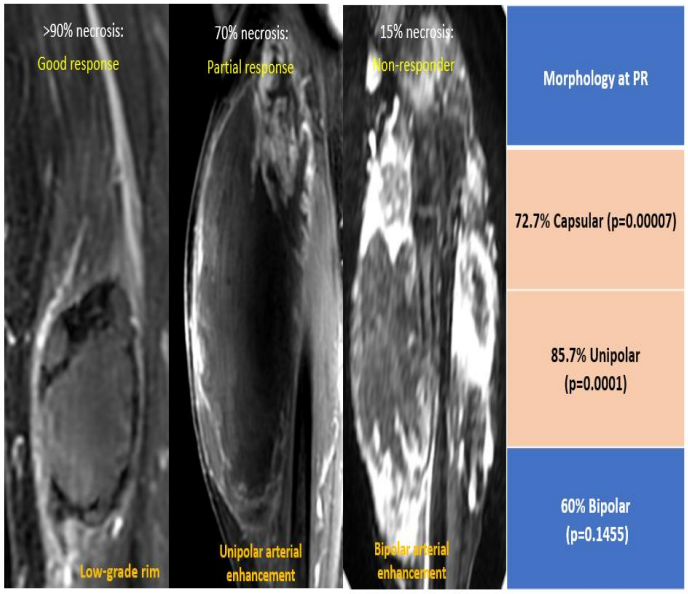


Figure 8. Perfusion-weighted Imaging (PWI)/ Dynamic contrast-enhanced (DCE) can assist in the morphologic stratification by the degree of UPS necrosis as a low-grade rim, unipolar arterial enhancement, and bipolar arterial enhancement predominantly.

(P 357) APPARENT DIFFUSION COEFFICIENT (ADC) HIGH-ORDER RADIOMICS OF BASELINE AND POST-TREATMENT ADVANCED MRI OF EXTREMITY SOFT-TISSUE NON-MYXOID UNDIFFERENTIATED PLEOMORPHIC SARCOMA.

Raul F. Valenzuela, M.D[1]; Elvis Duran Sierra, PhD[1]; Behrang Amini, M.D, PhD[2]; Mathew Antony, M.D[3]; John E. Madewell, M.D[1]; Colleen Costelloe, M.D[3]; William A. Murphy, Jr., M.D[4], (1) MD Anderson Cancer Center, Houston, TX, (2) MD Anderson cancer Center, Houston, TX, (3) MD Anderson cancer center, Houston, TX, (4) M.D Anderson cancer center, Houston, TX

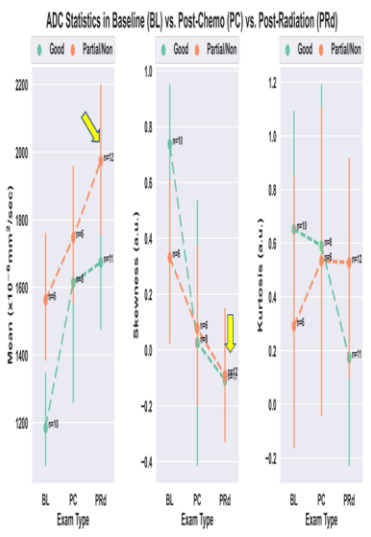
Objective: To determine the value of ADC radiomic high-order features from advanced MRI with Diffusion Weighted Imaging (DWI), in order to characterize UPS treatment response.

Methods: This retrospective IRB-authorized study included 26 extremity non-myxoid UPS patients who underwent imaging and surgical resection from February 2021-October 2022. Volumetric tumor segmentation from ADC measurements was obtained at multiple time points: Baseline (BL), Post-Chemotherapy (PC), and Post-Radiation (PRd).

Treatment-induced tumor necrosis (TIN) in surgical specimens was use to separate patients into Good Responders (GR: TIN>90%, thirteen patients), Partial-Responders (PR: 89-31%, eight patients), and Non-Responders (NR: < 30%, five patients).

Results: High-order radiomic features were extracted with a 95% confidence interval for 107 radiomic features, comparing 8 GR vs. 7 PR-NR at BL. Two-tailed non-parametric (Wilcoxon rank-sum) statistical tests were performed on the top 10 radiomic features. Statistical analysis displayed statistically significant differences ($p< 0.05$) in 3 BL features: Skewness, Median, and root-mean-squared. A linear support vector machine (SVM) model with an L-1 penalty was trained to distinguish GR vs. PR-NR and demonstrated the ten most relevant radiomic features. Significant differences ($p< 0.05$) seen at PC: Difference Entropy and Difference Variance. Significant differences ($p< 0.05$) at PRd: 10-percentile, Median, and mean.

Conclusion: Combining statistical significance analysis and linear SVM modeling, we found that the 10-Percentile and Median at PRd and Skewness at BL are strongly associated with good response in non-myxoid UPS.



		ADC		
		Mean	Skewness	Kurtosis
Good Responders	PC vs. BL	+36% (p=0.0545)	-96% (p=0.0545)	-9% (p=0.6964)
	PRd vs. BL	**+41% (p=0.0017)**	**-115% (p=0.0006)**	-73% (p=0.1131)
Partial/Non-Responders	PC vs. BL	+12% (p=0.3939)	-77% (p=0.1796)	+83% (p=0.5887)
	PRd vs. BL	+26% (p=0.0319)	-127% (p=0.0528)	+81% (p=0.4936)

Figure 2. The left panel shows the Apparent Diffusion Coefficient (ADC) changes of Non-Myxoid Undifferentiated Pleomorphic Sarcoma at baseline, post-chemotherapy (PC), and post-radiation (PRd) in good vs partial/non-responders. The right panel shows that the most significant ADC changes were in Good responders in post-radiation Vs Baseline with a 41% increased ADC mean and 115% reduction of the skewness. While considering post-radiation alone, no significance was found separating Good responders Vs Poor responders/non-responders. This highlights the importance of Advanced Bone and Soft Tissue Imaging (ABASTI) baseline.

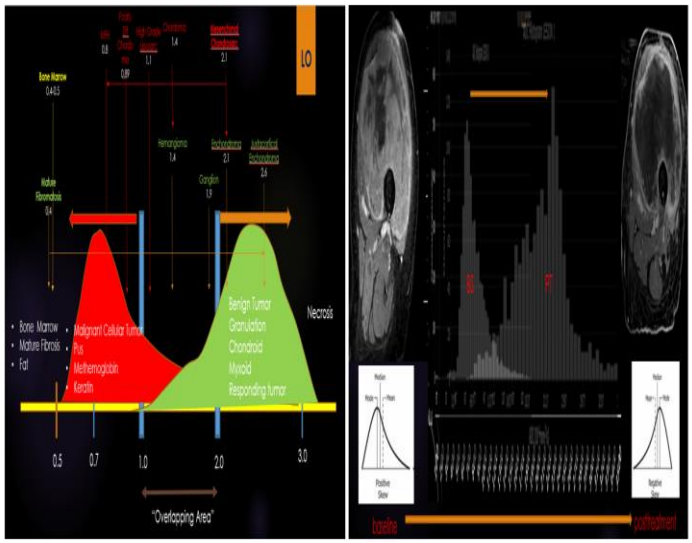


Figure 1. Apparent Diffusion Coefficient (ADC) which is used to measure tumor cellularity can also be used to evaluate therapeutic response. The left panel shows the various entities that radiologists come across and their respective range of ADC values. The right panel shows a pre-therapeutic contrast-enhanced MRI of the left thigh with a predominance of solid internal enhancement, associated with a left-sided ADC histogram (positive skewness) white a post-therapeutic contrast-enhanced MRI demonstrating markedly diminished sold enhancement and a right-sided ADC histogram (negative skewness).

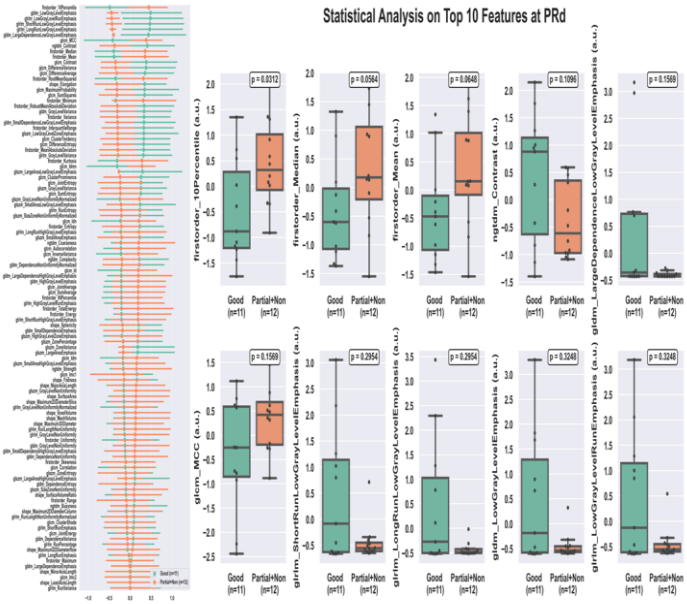


Figure 3. UPS ADC analysis at post-radiation (PRd): Scatter plot (left panel) showing the mean and 95% confidence interval for each of the 107 radiomic features extracted comparing good responders vs. partial/non-responders at post-radiation. Features are sorted from top to bottom based on the difference between group means. Boxplots of top 10 radiomic features (right panel) comparing good vs. partial/non-responders. P-values resulted from two-tailed non-parametric Wilcoxon rank-sum tests.

(P 358) CONTRAST-ENHANCED SUSCEPTIBILITY IMAGING (CE-SWI) MORPHOLOGIC, FIRST & HIGH-ORDER RADIOMIC FEATURES VALUE IN ASSESSING TREATMENT RESPONSE IN EXTREMITY SOFT-TISSUE UNDIFFERENTIATED PLEOMORPHIC SARCOMA

Raul F. Valenzuela, M.D[1]; Elvis Duran Sierra, PhD[1]; Behrang Amini, M.D, PhD[2]; Mathew Antony, M.D[3]; John E. Madewell, M.D[1]; Colleen Costelloe, M.D[3]; William A. Murphy, Jr., M.D[4], (1) MD Anderson Cancer Center, Houston, TX, (2) MD Anderson cancer Center, Houston, TX, (3) MD Anderson cancer center, Houston, TX, (4) M.D Anderson cancer center, Houston, TX

Objective: Undifferentiated pleomorphic sarcomas (UPS) are the largest soft tissue sarcomas (STS) subgroup. Post-therapeutically demonstrates hemosiderin deposition, granulation tissue formation, fibrosis, and calcification.

To determine the value of morphologic patterns and radiomic high-order features from advanced MRI with Contrast-enhanced Susceptibility Imaging (CE-SWI) to characterize undifferentiated pleomorphic sarcoma (UPS) treatment response.

Methods: This retrospective IRB-authorized study included 26 extremities soft-tissue non-myxoid UPS patients with imaging and resection performed from February 2021-October 2022. Volumetric tumor segmentation from CE-SWI was obtained at multiple time points: Baseline (BL), Post-Chemotherapy (PC), and Post-Radiation (PRT).

The percentage of treatment-induced tumor necrosis (TIN) in surgical specimens separated cases into Good Responders (GR: TIN>90%, 13 patients), Partial-Responders (PR: 89-31%, eight patients), and Non-Responders (NR: < 30%, five patients).

Results: SWI patterns: "Complete ring" was observed in 82% of Good Responders GR) (p=2.1x10-6). "Incomplete ring" in 50% of Partial Responders (PR) (p=0.1455) and a "Globular pattern" in 60%. Of Non-Responders (NR).

First-order Radiomic features: A decrease in CE-SWI-mean from 700 at BL to 100 at PC was observed, due to treatment-induced bleeding T2* hypointensity.

High-order radiomic features were extracted with a 95% confidence interval for 107 radiomic features, comparing "complete ring" (10 patients) vs. other SWI patterns (12 patients). Two-tailed non-parametric (Wilcoxon rank-sum) statistical tests were performed on the top 10 radiomic features.
Comparing GR (n=11) vs. PR-NR (n=11) at PRT: Large Dependence Emphasis, Dependence Variance, Maximum Probability, Run Percentage, Large-Dependence-Low-Gray-Level-Emphasis, and Short Run Emphasis.

Conclusion: Advanced MRI with CE-SWI is a valuable tool for UPS treatment assessment, demonstrating hemorrhage patterns as biomarkers of treatment effect. The high-order radiomic analysis found six texture features separating GR vs. PR/NR. CE-SWI "complete ring" morphology was the most common and statically associated pattern observed in GR at PRT.

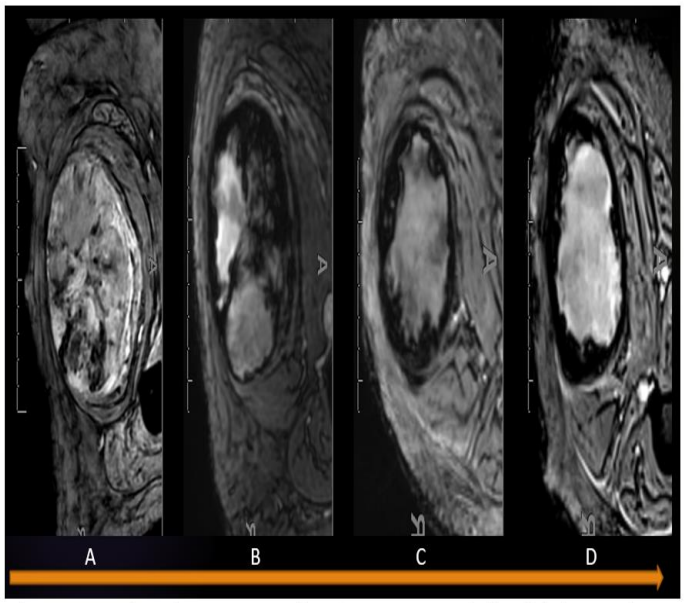


Figure 1. **Tumor hemorrhage as a Biomarker of Response:** SWI of right thigh demonstrating an undifferentiated pleomorphic sarcoma (UPS) at different stages during therapy. (A) Baseline, (B) Post-chemo initial, (C) post chemo final, (D) Post-radiation: 99% necrosis on the histologic specimen.

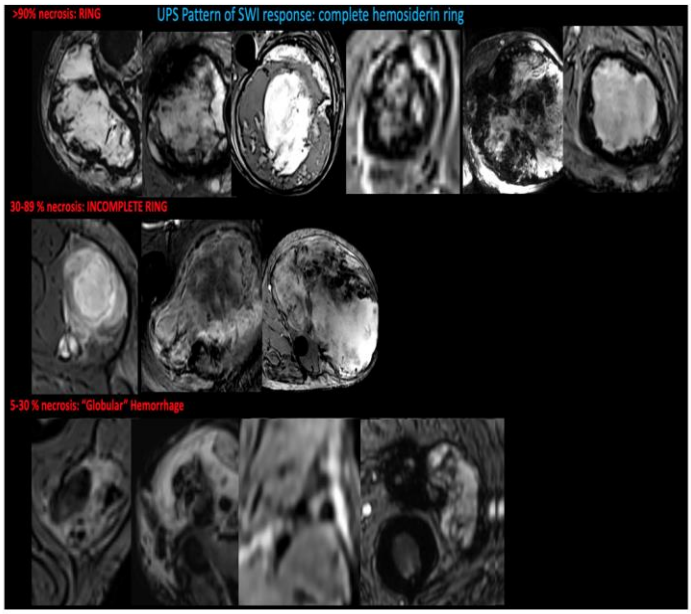


Figure 2. Tumor Hemorrhage: Using SWI-CE, these patterns are seen in Undifferentiated pleomorphic sarcoma (UPS) during treatment. The top line demonstrates a "complete ring", the middle line demonstrates an "incomplete ring", and the bottom line demonstrates "globular hemorrhage".

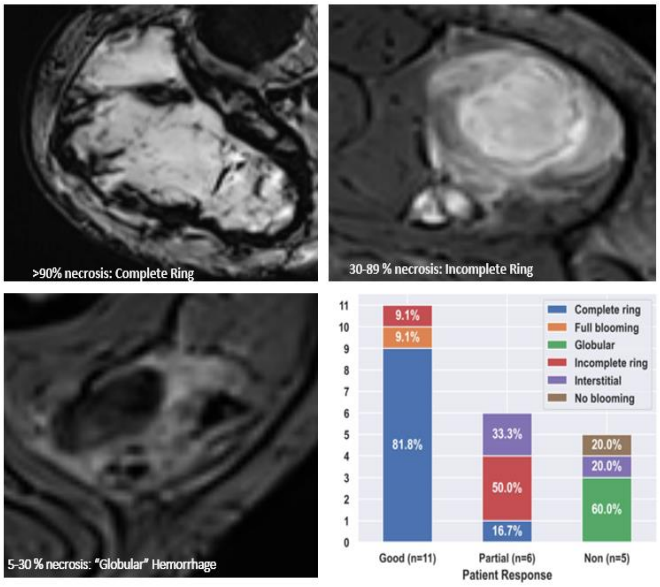
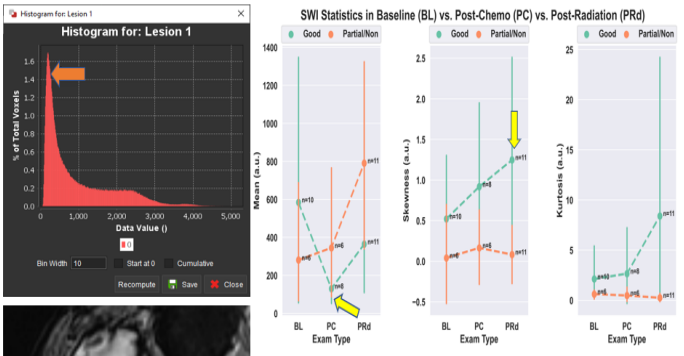


Figure 3. "Complete ring" in good responders was observed in 82% of patients (p=0.000002), "Incomplete ring" in partial responders was observed in 50% of patients (p=0.1562) and a "globular" pattern in non-responders was observed in 60% of patients (p=0.1455).



			SWI		
			Mean	Skewness	Kurtosis
Good Responders	PC vs. BL		-78% (p=1.0)	+76% (p=0.4236)	+25% (p=1.0)
	PRd vs. BL		-38% (p=0.8602)	+139% (p=0.3072)	+295% (p=0.9719)
Partial/Non-Responders	PC vs. BL		+23 (p=0.5887)	+296% (p=1.0)	-22% (p=0.3939)
	PRd vs. BL		+182% (p=0.2561)	+99% (p=0.7248)	-59% (p=0.2275)

Figure 4. In good responders, At Baseline the SWI-CE mean decreased by 78% after chemotherapy due to treatment-induced bleeding T2* hypo intensity. It was also observed that the Skewness increased 139% at post radiation.

(P 359) ROLE OF PERFUSION WEIGHTED IMAGING WITH DYNAMIC CONTRAST ENHANCED (PWI/DCE) FOR THE TREATMENT ASSESSMENT OF EXTREMITY SOFT-TISSUE NON-MYXOID UNDIFFERENTIATED PLEOMORPHIC SARCOMA

Raul F. Valenzuela, M.D[1]; Elvis Duran Sierra, PhD[1]; Behrang Amini, M.D, PhD[2]; Mathew Antony, M.D[3]; John E. Madewell, M.D[1]; Colleen Costelloe, M.D[3]; William A. Murphy, Jr., M.D[4], (1) MD Anderson Cancer Center, Houston, TX, (2) MD Anderson cancer Center, Houston, TX, (3) MD Anderson cancer center, Houston, TX, (4) M.D Anderson cancer center, Houston, TX

Objective: Undifferentiated pleomorphic sarcomas (UPS) are the largest subgroup of soft tissue sarcomas. Post-therapeutically demonstrate hemosiderin deposition, granulation tissue formation, fibrosis, and calcification. The aim of this study is to determine the value of advanced MRI with Perfusion-WI (PWI/DCE) in evaluating UPS treatment response.

Methods: A retrospective IRB-authorized study included 26 patients with extremity non-myxoid UPS who underwent pre-surgical imaging and surgical resection from February 2021-October 2022. Treatment-induced tumor necrosis (TIN) in surgical specimens separated cases as Good Responders (GR: TIN>90%, 13 patients), Partial-Responders (PR: 89-31%, 8 patients), and Non-Responders (NR: < 30%, 5 patients). Volumetric tumor segmentation was performed on routine advanced MRI at the Baseline (BL), Post-Chemotherapy (PC), and Post-Radiation (PRT) pre-surgical time points. DCE-PWI images were manually contoured using the MIM-licensed software, and the final contours were exported as DICOM files. A Python-based software application developed in our institution automatically extracted 7 semi-quantitative perfusion kinetic parameters. Statistical analyses compared good vs. partial/non-responders.

Results: At post-radiation (PR), 72.7% of GR displayed Capsular morphology (p=0.00007), and

100% displayed a TIC-type 2 (p=0.00002). 85.7% of partial responders showed Unipolar morphology (p=0.0001), and 71.4% expressed a TIC type 5 (p=0.0662). 60% of non-responders showed Bipolar morphology (p=0.1455), and 80% expressed a TIC type 5 (p=0.0742). The statistical analysis comparing the 7 semi-quantitative metrics separating good vs. partial/non-responders demonstrated statistical significance (p=0.0164) for wash-in rate (WiR).

Conclusion: UPS treatment assessment can rely on whole tumor PWI/DCE time-intensity curve (TIC), semi-quantitative and morphologic analysis, where TIC II, capsular enhancement, and high WiR correlate with successfully treated sarcomas with 90% or more TIN. DCE qualitative TIC and combined morphologic and semi-quantitative kinetic analysis demonstrate significant potential to serve as predictors of response in UPS.

Clinical Relevance: Perfusion Weighted Imaging (PWI) with Dynamic Contrast-Enhanced (DCE) imaging can assess treatment response in non-myxoid undifferentiated pleomorphic sarcoma.

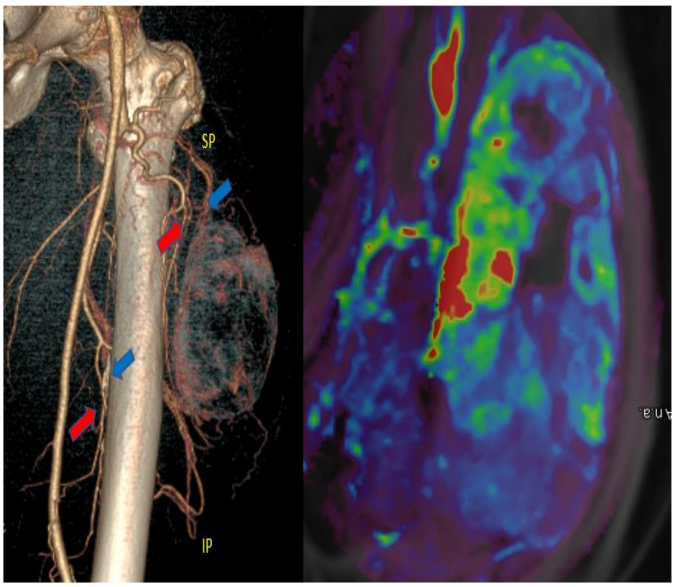


Figure 1. Sarcoma Organ model of Undifferentiated pleomorphic sarcoma: On the left, we see a superior arterial pole equatorial necrosis with an inferior venous pole. On the right, We see oxygen and flow gradients demonstrating a central necrosis and polar residual disease.

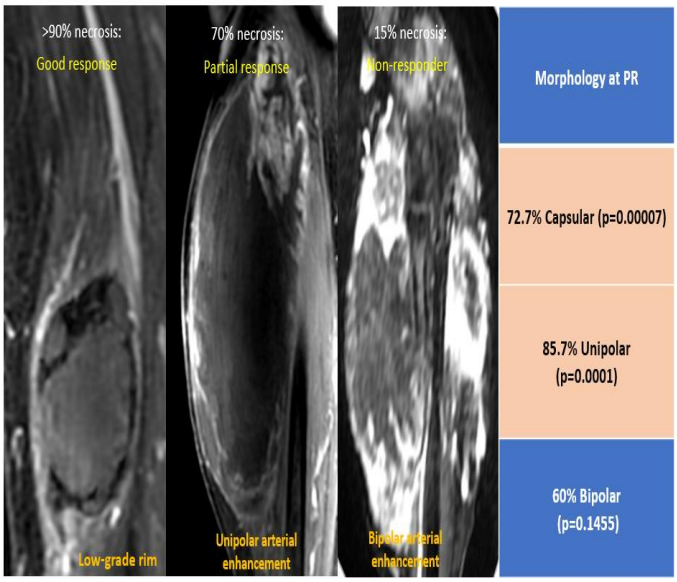
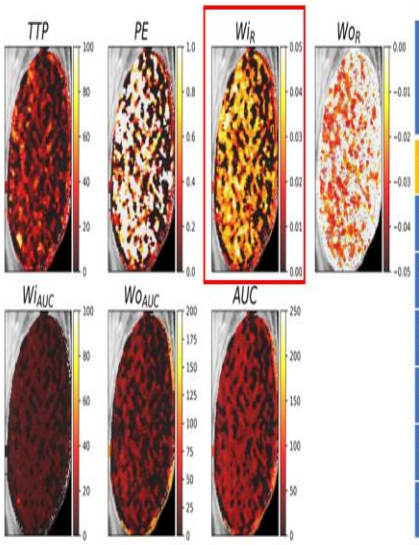
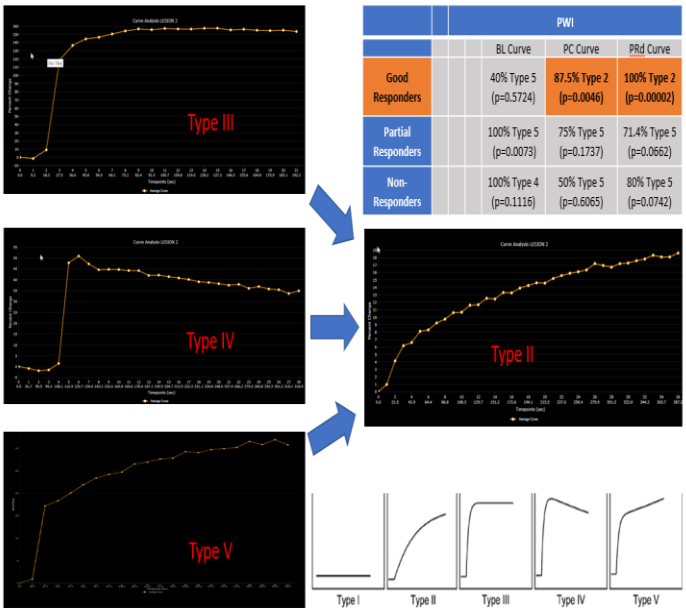


Figure 2. Perfusion-weighted Imaging (PWI)/ Dynamic contrast-enhanced (DCE) can assist in the morphologic stratification by the degree of UPS necrosis as a low-grade rim, unipolar arterial enhancement, and bipolar arterial enhancement.



Kinetic Parameters at Post-Radiation	P-Value
WiR	0.0164
WoR	0.1096
PE	0.1962
WiAUC	0.1962
TTP	0.4417
WoAUC	0.7119
AUC	0.8535

Figure 3. Tumor Vascularity was analyzed by the results of Perfusion weighted Imaging (PWI)/ Dynamic contrast-enhanced (DCE) patterns. Seven Kinetic maps and their corresponding p-values comparing good vs. partial/non-responders at post-radiation are shown. WiR decreased by 171% in good responders (p=0.0164) relative to partial/non-responders, showing the highest statistical association with response.



	PWI		
	BL Curve	PC Curve	PRd Curve
Good Responders	40% Type 5 (p=0.5724)	87.5% Type 2 (p=0.0046)	100% Type 2 (p=0.00002)
Partial Responders	100% Type 5 (p=0.0073)	75% Type 5 (p=0.1737)	71.4% Type 5 (p=0.0662)
Non-Responders	100% Type 4 (p=0.1116)	50% Type 5 (p=0.6065)	80% Type 5 (p=0.0742)

Figure 4. Pre-therapeutic and Post-Therapeutic Time Intensity Curves of Cellular STS/Non-Myxoid UPS: The left three panels show Type III, IV, or V Kinetic curves which are associated with untreated UPS. Following successful therapy with greater than 90% necrosis, the kinetic curve transitions to Type II as seen in the right middle panel.

(P 360) **TP53 ALTERATIONS IN SARCOMAS: AN ANALYSIS FROM THE AACR-GENIE DATABASE**

Jason Roszik, PhD, MBA[1]; Ryan A. Denu, MD, PhD[1]; Elise F. Nassif, MD[2]; Michael S. Nakazawa, MD, PhD[1]; Andy Livingston, MD, MS[3]; Kadir C. Akdemir, PhD[1]; Ravin Ratan, MD[3]; Alexander J. Lazar, MD, PhD[3]; Wei-Lien Wang, M.D[3]; Roberto Pestana, MD[4]; Ahsan S. Farooqi, MD PhD[5]; Christina Roland, MD, MS[3]; Emily Z. Keung, MD, AM, FACS[3]; Vinod Ravi, MD, MS, MBA[3]; Neeta Somaiah, MD[3]; Shreyaskumar Patel, MD[3]; Anthony P. Conley, MD[1], (1) MD Anderson Cancer Center, Houston, TX, (2) University of Texas MD Anderson Cancer Center, Houston, TX, (3) The University of Texas MD Anderson Cancer Center, Houston, TX, (4) Hospital Israelita Albert Einstein, Sao Paulo, Sao Paulo, Brazil, (5) MD Anderson Cancer Center, Sugar Land, TX

Objective: TP53 is the most commonly mutated gene in cancer, and it plays a significant role in sarcomas. Various structural alterations of TP53 (somatic and germline) have been observed in sarcomas. Effects on disease-free survival and responsiveness with certain therapeutics have been reported in sarcomas(anthracyclines).[PMID: 34282771] In addition, the development of p53 reactivators in preclinical studies and early phase clinical trials are demonstrating signs of efficacy. [PMID: 35948006] The aim with this study is to perform a comprehensive analysis on the frequency, mutation type, histologic distribution, and evaluation of other alterations associated with TP53-mutated sarcomas.

Methods: GENIE cohort (version 13.1) was evaluated for de-identified sarcoma files, which include limited clinical information and genomic data. cBioPortal and R software was utilized for data visualization and mutation mapping. GraphPad Prism (version 9) and Tableau Desktop 8.1

were utilized for descriptive statistics. OncoKB (oncokb.org) was utilized to annotate the type and function of gene alterations and to determine actionability of each mutational event. Gene ontology analysis (GOA) was performed on genes with mutations detected at > 2.5% frequency with PANTHER 17.0 (pantherdb.org). Fisher's exact test was performed for enrichment analysis.

Results: 5,222 sarcoma samples from 4,875 patients were identified. 251 patients had 2 or more samples each (5.1%). Primary tumors represented 3,298 of sample (63%) and metastatic sites accounted for 1,791 of samples (34%). Of these, 2,092 samples harbored TP53 mutations. The most common TP53 mutations in sarcomas included p.R175H (n=80 patients), p.R248Q (n=72), p.R273H (n=63), p.R273C (n=51), p.R248W (n=49), p.Y220C (n=42), and p.R342* (n=40). With exception of p.R342*, the other most common hotspot mutations occur in the p53 DNA binding domain. By OncoKB, p.R175H, p.R273H, p.Y220C, and p.R342* were considered oncogenic, loss-of-function; p.R248Q, p.R273C, and p.R248W were only likely oncogenic. The p.Y220C variant was associated with more TP53 CNAs compared to the other most common hotspot TP53 mutations. Uterine carcinosarcomas presented the most common histology across these 7 hotspot mutations. Missense mutations (n=1430), frame shift deletions (n=264), nonsense mutations (n=249), and splice site variants (n=149) were the most common mutation types. Compared to wild type TP53 (median 3, range 1 – 293), sarcomas with TP53 alterations (median 5, range 1 – 120, p< 0.0001) harbored a higher mutation count. A GOA demonstrated a >100-fold over enrichment for telomerase activities.

Conclusion: This dataset represents the largest sample of TP53 alterations in sarcomas. The most common hotspot mutations of TP53 were oncogenic with loss-of-functionality. TP53 mutated sarcomas had higher mutation counts than sarcomas without TP53 alterations. Regulation of telomerase activities were over-represented by GOA.

(P 361) MOLECULAR CHARACTERIZATION OF SOLITARY FIBROUS TUMORS BY ANATOMIC SITE AND NAB2::STAT6 FUSION VARIANT TYPE

Priscila Barreto coelho, MD[1]; Andrew Elliott, MD PhD[2]; Steven Bialick, DO[3]; Brandon E. Rose, MD, MPH[4]; Matthew Oberley, MD PhD[2]; Andreas Seeber, MD PhD[5]; Margaret von Mehren, MD[6]; César Serrano, MD, PhD[7]; Andrew Rosenberg, MD[8]; Jaime F. Modiano, VMD PhD[9]; Andrea P. Espejo-Freire, MD[10]; Vaia Florou, MD MS[11]; Kirsten Leu, N/A, MD[12]; Bradley DeNardo, MD[13]; Don S. Dizon, MD[14]; Galina Lagos, MD[15]; Sosipatros A. Boikos, MD[16]; Moh'd Khushman, MD[17]; Atrayee Basu Mallick, MD[18]; Robert G. Maki, MD PhD[19]; Aditi Dhir, MD[20]; Vasileios Stathias, PhD[21]; Emily Jonczak, MD[22]; Jonathan Trent, MD, PhD[23]; Gina Z. D'Amato, MD[21], (1) University of Miami, Miami, FL, (2) Caris Life Sciences, Phoenix, AZ, (3) Sylvester Comprehensive Cancer Center, University of Miami, Deerfield Beach, FL, (4) University of Miami and Jackson Health Systems, Miami Beach, FL, (5) Medical University of Innsbruck, Innsbruck, Tirol, Austria, (6) Fox Chase Cancer Center, Philadelphia, PA, (7) Medical Oncology Department, Hospital Universitario Vall d'Hebron, Vall d'Hebron Institute of Oncology, Barcelona, Spain, Barcelona, Catalonia, Spain, (8) Sylvester Comprehensive Cancer Center, MIAMI, FL, (9) University of Minnesota, Minneapolis, MN, (10) University of Minnesota, MInneapolis, MN, (11) Huntsman Cancer Institute University of Utah, SALT LAKE CITY, UT, (12) Nebraska Cancer Center, Omaha, NE, (13) Lifespan, Providence, RI, (14) Brown

University, Providence, RI, (15) Brown University / Lifespan Cancer Institute, Providence, RI, (16) Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, (17) Washington University in St. Louis / Siteman Cancer Center, St. Louis, MO, (18) Thomas Jefferson University Hospital, Philadelphia, PA, (19) Memorial Sloan Kettering Cancer Center; Professor, Weill Cornell Medical College, New York, NY, (20) University of Miami Miller School of Medicine, Miami, FL, (21) Sylvester Comprehensive Cancer Center, Miami, FL, (22) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL, (23) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL

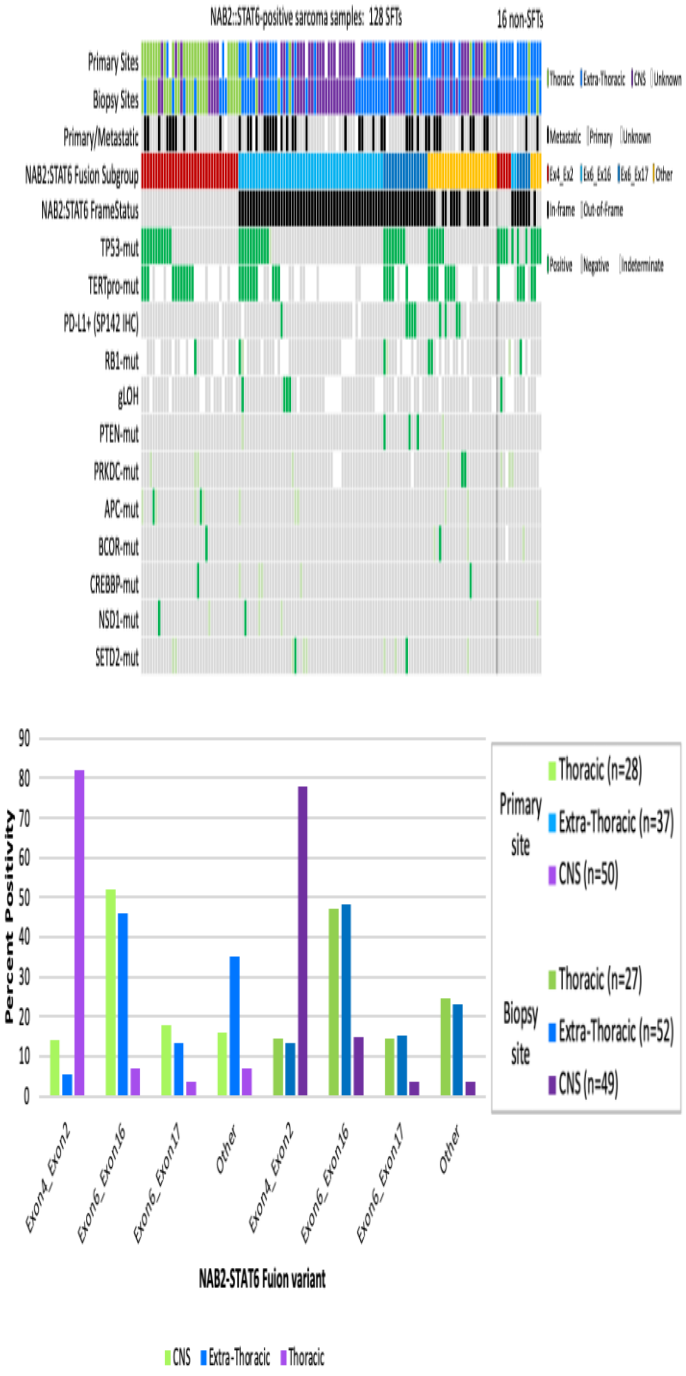
Objective: Solitary Fibrous Tumor (SFT) is a rare mesenchymal tumor with an incidence of 1 new case/per million people/year. Advances in immunohistochemistry and molecular diagnostics identified STAT6 nuclear protein expression and the NAB2::STAT6 fusion gene as more precise tools for SFT diagnosis. Still, since the fusion transcript driver's first description in 2013, few new insights have been brought into critical molecular events in SFT. The prognosis of SFT patients is associated with anatomic localization, and an independent prognostic impact of the NAB2::STAT6 fusion variant has been suggested. We aimed with our study to evaluate the molecular landscape of SFT patients and the impact of the NAB2::STAT6 fusion variant type on the largest cohort reported to date on this rare type of sarcoma.

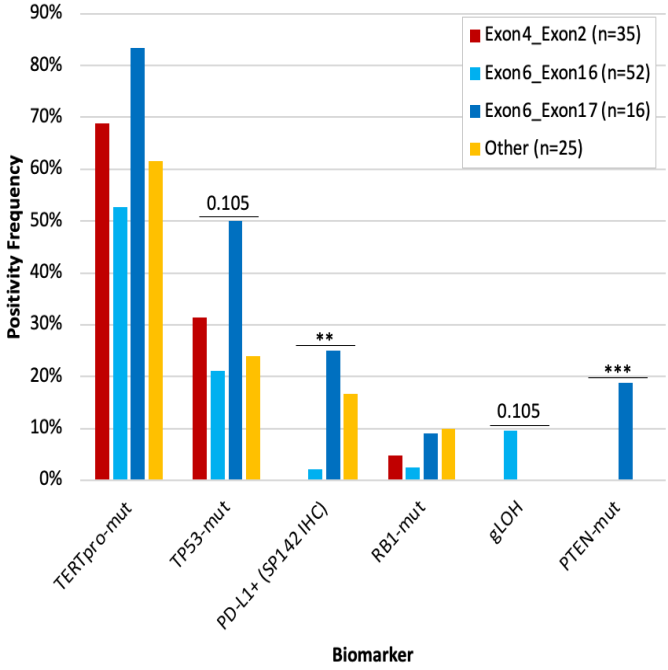
Methods: This is an institutional review board approved, retrospective analysis of next-generation DNA (targeted, n=24, or whole exome, n=104) and RNA (whole transcriptome, n=128) sequencing data for patient tumors submitted to Caris Life Sciences (Phoenix, AZ). Included in this analysis were 128 samples with a reported diagnosis of SFT and NAB2::STAT6 fusion transcript detected, along with 16 NAB2::STAT6 fusion-positive samples with non-SFT histology reported. NAB2::STAT6 fusion variant were categorized based on associated fusion junction exons. Tissue biopsy sites were classified as thoracic (T), extra-thoracic (ET), or CNS according to anatomic location. Tumor microenvironment (TME) immune cell population abundance estimated by using MCP-counter (Becht, 2016). Statistical significance was determined using X2 and Mann-Whitney U tests.

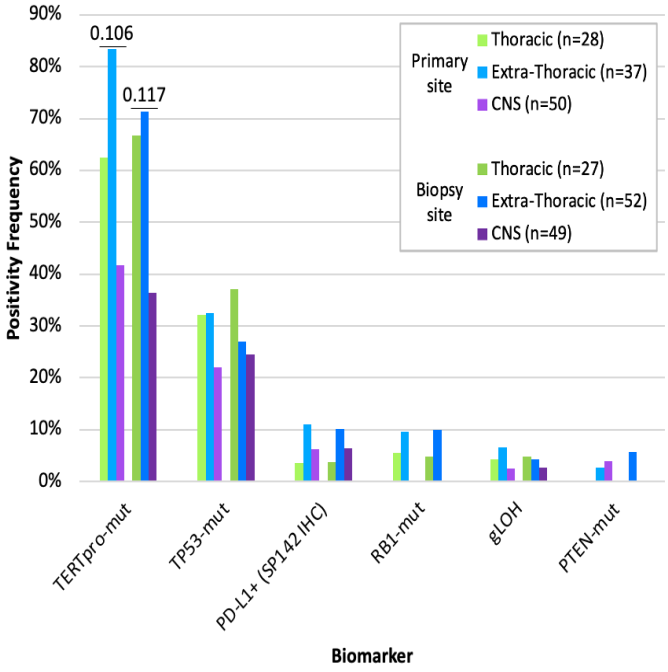
Results: SFT samples were comprised of equal proportions of male and female patients (50%, n=64 each), with a median age of 61 years, and 59% (n=76) were metastatic biopsies (8 unknown). The most common NAB2::STAT6 fusion transcripts were Exon6::Exon16 (Ex6::Ex16; 41%, n=52), Ex4::Ex2 (27%, n=35), and Ex6::Ex17 (13%, n=16), with several other variant transcripts with low prevalence of fused exons ('other'; 19%, n=25). Thoracic SFTs frequently harbored Ex4::Ex2 variant transcripts compared to non-thoracic tumors (78%, n=21; ET: 13%, n=7; CNS 14%, n=7; p< 0.001)t. SFTs from ET and CNS sites had similar enrichment of Ex6::Ex16 variants (ET: 48%, n=25; CNS: 47%, n=23; T: 15%, n=4; p< 0.001) and greater proportions of tumors with other rare variant transcripts compared to T sites (ET: 23%, n=12; CNS: 24%, n=12; T: 4%, n=1; p< 0.001). Aside from the NAB2::STAT6 fusion, tumor suppressor genes TP53 (28%, n=36), RB1 (5.4%, n=5), and PTEN (2.3%, n=3) were among the few recurrent genomic alterations, as well as TERT promoter mutations (63%, n=34/54 tested). Ex6::Ex17 variant samples were associated with greatest frequency of TP53 mutations (50% vs 21-31% for other fusion variants; p=0.101) and exclusively associated with PTEN mutations (19% vs 0%; p< 0.001). PDL1+ expression was associated with Ex6::Ex17 (25% vs 17% 'other', 2% Ex6::Ex16, and 0% Ex4::Ex2; p=0.002), yet Ex6::Ex17 TMEs had relatively low abundance of pro-inflammatory cell types, which were most abudance in Ex6::Ex16 tumors. SFTs from CNS-related sites had a trend toward a lower prevalence of TERT promotor mutations compared to non-CNS (36% vs 67-71%; p=0.12).

Conclusion: We molecularly characterized the largest known cohort of real-world SFT patient samples and identified new relationships between genomic alterations, tumor site, and

NAB2::STAT6 fusion variant type. While confirming the association of thoracic SFTs with Ex4::Ex2 variants, we find that Ex6::Ex16 and Ex6::Ex17 variant subgroups have unique molecular profiles, including a notable difference in PDL1+ frequencies, which warrant further investigation of these fusion variant SFT subpopulations in order to develop new therapeutic approaches including the potential for immune checkpoint inhibitors.




90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Positivity Frequency
Exon4_Exon2 (n=35)
Exon6_Exon16 (n=52)
Exon6_Exon17 (n=16)
Other (n=25)
0.105
**
0.105
***
TERTpro-mut
TP53-mut
PD-L1+ (SP142 IHC)
RB1-mut
gLOH
PTEN-mut
Biomarker


90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Positivity Frequency
0.106
0.117
Primary site
Thoracic (n=28)
Extra-Thoracic (n=37)
CNS (n=50)
Biopsy site
Thoracic (n=27)
Extra-Thoracic (n=52)
CNS (n=49)
TERTpro-mut
TP53-mut
PD-L1+ (SP142 IHC)
RB1-mut
gLOH
PTEN-mut
Biomarker

(P 362) IDENTIFICATION OF A NOVEL INTERACTOR OF ENDOGENOUS SS18-SSX THROUGH MASS SPECTOMETRY-BASED ANALYSIS IN SYNOVIAL SARCOMA CELLS

Ainiah Rushdiana Raquib, B.Sc[1]; Torsten O. Nielsen, MD, PhD[2], (1) UBC, Vancouver, BC, Canada, (2) Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Objective: The role of the fusion oncoprotein SS18-SSX in synovial sarcoma has been studied extensively to understand how one mutant protein is able to cause and sustain oncogenesis. Experiments using mass spectrometry to analyse interactors of SS18-SSX have mostly relied on cell line models engineered to tag the protein for pulldown experiments, resulting in discovery of its association with a diverse set of proteins including HDAC, ATF2, TLE1, KDM2B and the mammalian SWI/SNF (complex). The lack of an antibody specific to SS18-SSX has limited these studies to epitope-tagged single cell-line models. In the current study, immunoprecipitation of the native SS18-SSX protein was performed in multiple synovial sarcoma cell lines with subsequent analyses of the interacting proteins through tandem mass spectrometry. The study aims to identify novel interactors of the synovial sarcoma fusion oncoprotein in endogenous cellular backgrounds.

Methods: Whole cell lysates were collected from six synovial sarcoma cell lines which have previously been shown to express the fusion oncoprotein using a commercially available SS18-SSX fusion specific antibody. An osteosarcoma cell line was included as a negative control. Dynabeads were used to perform immunoprecipitation (IP) with the SS18-SSX antibody and a control rabbit IgG antibody, in triplicates. Samples were digested with trypsin and processed for tandem mass tag labelling of peptides and subsequent analysis on an Orbitrap Fusion Tribrid MS platform. Peptides were analysed using Proteome Discoverer Software, and reporter abundance was averaged across triplicate runs.

Results: Protein abundance was compared in each cell line and log2 fold change values calculated for proteins enriched in the SS18-SSX IP group over the control IgG group. Comparison of the top hits in all synovial sarcoma samples identified a canonical PRC1 complex member, CBX4, as a positive hit in 4 out of 6 cell lines. As part of the canonical PRC1 complex, CBX4 utilises its chromodomain to recognise the epigenetic H3K27me3 mark on histones to mediate transcriptional repression. Furthermore, CBX4 also functions in a dual role as an E3 SUMO ligase within the SUMOylation pathway, whose other members (SUMO2, UBC9) have been shown to be enriched in synovial sarcoma lineages (DepMap). Co-IP of SS18-SSX followed by western blot detection showed CBX4 to be present in all 6 cell lines and immunofluorescence imaging confirmed the co-localisation of SS18-SSX with CBX4 in the nucleus.

Conclusion: Using the fusion-specific antibody for immunoprecipitation of the endogenous SS18-SSX protein helps to detect common interactors amongst different synovial sarcoma cell lines, and uncover new pathways and roles for the protein in sarcomagenesis. We uncover a novel interaction of SS18-SSX with CBX4, a canonical PRC1 member involved in transcriptional repression. This interaction presents itself as a potential druggable target in synovial sarcoma with the use of existing molecules that can inhibit CBX4 function.

(P 363) **B-CATENIN MUTATIONS AND TRANSCRIPTOMIC REPROGRAMMING IN DESMOID FIBROMATOSIS (DF)**

Stefano Percio, PhD[1]; Laura Bergamaschi, MSc[2]; Loris De Cecco, PhD[3]; Armando Giuseppe Licata, n/a[1]; Federica Perrone[1]; Valentina Guarino, n/a[1]; Sandro Pasquali, MD, PhD[1]; Nadia Zaffaroni, PhD[1]; licia Rivoltini, MD, PhD[1]; Alessandro Gronchi, MD, FSSO[4]; Viviana Vallacchi, PhD[1]; Chiara Colombo, MD[5], (1) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (2) Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Milan, Lombardia, Italy, (3) Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Lombardia, Italy, (4) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (5) Department of Surgery, Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy

Objective: Active surveillance (AS) is the first-line strategy for the vast majority of DF patients. One in four patients experiences spontaneous regression as first event and an additional 30% develop a tumor regression after an early progression. However, approximately 35% may require active treatment for meaningful clinical tumor progression. We previously reported that DF surgical specimens of patients who underwent surgery for DF have an inflammatory phenotype when harboring T41A-mutated β-catenin (PMID: 28627792). However, there is a limited knowledge of biology and immune contexture of DF and biomarkers to predict the clinical course during AS are lacking. In this study, we deeply investigated transcriptomic profile of patients with-CTNNB1 mutated DF exploiting a prospective clinical trial on AS in patients with a primary diagnosis of DF.

Methods: A prospective study was conducted at our Institution and enrolled 56 patients affected by primary sporadic DF. CTNNB1 mutation status of DF biopsies was determined by Sanger sequencing. CTNNB1-targeted DNA sequencing with NGS approaches was performed in case of WT DF at Sanger sequencing. FFPE biopsies at DF diagnosis had been prospectively collected and 39 biopsies from patients with CTNB1-mutated DF were suitable for RNA extraction and sequencing with RNA-Seq technique. Functional analysis of deregulated genes was obtained by Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA).

Results: In this clinical study, we observed the following distribution of CTNNB1 mutations: S45F in 10 patients (31.3%), T41A in 22 (68.7%), S45P in 3 (7.6%), WT in 2 (5.1%), and “other” in 2 (5.1%). The computational analysis was then conducted to compare the more represented mutations (T41A vs S45F). Class comparison analysis at IPA identified 490 genes differentially expressed between S45F and T41A β-catenin-mutated DF biopsies ($p < 0.05$). T41A-mutated DF were positively enriched in gene sets related to inflammatory and immune system compared to S45F-mutated DF. Positive enrichment of ‘Epithelial Mesenchymal Transition’ (EMT) gene set was observed in S45F-mutated DF. At a median study follow-up of 18 months, 34% of patients experienced tumor progression, 25% tumor regression and 38% had stable disease as first event during AS according to RECIST criteria. A further class comparison analysis revealed 174 transcripts showing deregulated expression in DF from patients who experienced tumor progression (N=14) compared to DF from patients who had a tumor regression (N=9) during AS. A down-regulation of genes and molecular functions related to muscle activity and contractility and a negative enrichment of ‘Myogenesis’ gene set were observed in patients who experienced tumor progression.

Conclusion: In this preliminary analysis, we confirm that DF with T41A B-catenin gene

mutation has an ‘inflammatory phenotype’, as previously reported. We found an up-regulation of EMT genes in DF with S45F B-catenin gene mutation. A difference between gene expression in DF from patients who experienced either tumor progression or regression according to RECIST was observed. Further analyses evaluating association between DF β-catenin driven molecular alterations and patient outcome are ongoing.

(P 364) EVALUATION OF THE PREDICTIVE ROLE OF SYSTEMIC AND INTRATUMORAL IMMUNITY IN PATIENTS WITH LOCALIZED LIPOSARCOMAS, MYXOFIBROSARCOMAS AND UNDIFFERENTIATED PLEOMORPHIS SARCOMAS

Valentina Fausti, MD[1]; Silvia Vanni, PhD[2]; Eugenio Fonzi, n/a[3]; Alessandro De Vita, PhD[4]; Lorena Gurrieri, MD[1]; Nada Riva, MD[5]; Federica Pieri, MD[6]; Sofia Gabellone, n/a[2]; sebastiano Calpona, N/A, MD[3]; Nicoletta Ranallo, MD[3]; francesco De Rosa, MD[3]; Massimo Guidoboni, MD[3]; roberto Casadei, MD[7]; Marco Maraldi, MD[8]; Giorgio Ercolani, MD[9]; Davide Cavaliere, MD[9]; Carlo Alberto Pacilio, MD[9]; Devil Oboldi, MD[3]; Laura Ridolfi, MD[10]; Laura Mercatali, PhD[11], (1) Clinical and Experimental Oncology, Immunotherapy, Rare Cancers and Biological Resource Center, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", 47014 Meldola, Italy, Meldola, Emilia-Romagna, Italy, (2) Istituto Romagnolo per lo studio dei Tumori "Dino Amadori" IRST IRCCS, Preclinic and Osteoncology Unit, Meldola, Italy, Meldola, Emilia-Romagna, Italy, (3) Irst, Meldola, Emilia-Romagna, Italy, (4) Istituto Scientifico Romagnolo per lo studio dei Tumori "Dino Amadori", Preclinic and Osteoncology Unit, Meldola, Italy, Meldola, Emilia-Romagna, Italy, (5) Clinical and Experimental Oncology, Immunotherapy, Rare Cancers and Biological Resource Center, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Emilia-Romagna, Italy, (6) Pathology Unit, "Morgagni-Pierantoni" Hospital, Forlì, Emilia-Romagna, Italy, (7) Orthopedic Unit, Morgagni-Pierantoni Hospital, 47121 Forli, Italy, Forlì, Emilia-Romagna, Italy, (8) Ospedale Morgagni Pierantoni, forlì, Emilia-Romagna, Italy, (9) General and Oncologic Surgery, "Morgagni-Pierantoni" Hospital, Forlì, Emilia-Romagna, Italy, (10) IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" , Meldola, Emilia-Romagna, Italy, (11) Istituto Ortopedico Rizzoli IRCCS, Osteoncology- Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, Bologna, Italy ; Istituto Scientifico Romagnolo per lo studio dei Tumori "Dino Amadori", Preclinic and Osteoncology Unit, Meldola, Italy, Bologna, Emilia-Romagna, Italy

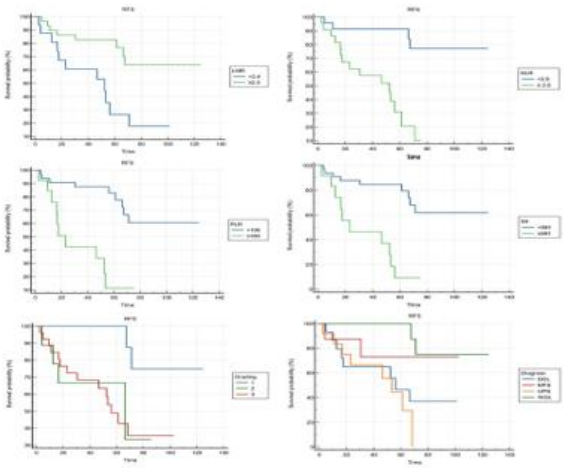
Objective: Achieving radical surgery still remains the only chance of recovery for most patients with soft tissue sarcoma. For this reason, identifying predictive factors of relapse that can guide best therapeutic planning remains an urgent need. This study aims to evaluate the ability of systemic and intratumoral immune status to predicte a disease relapse in patients affected by well and dedifferentiated liposarcoma (WDL, DDL), myxofibrosarcoma (MFS) and undifferentiated pleomorfic sarcoma (UPS) .

Methods: Forty-six patients that received surgery for localized WDL, DDL, MFS or UPS and followed up at the IRST Istituto Romagnolo per lo Studio dei Tumori (Meldola, Italy) from 2009 to May 2023 were retrospectively included in the study. Patients with both surgical specimen samples and presurgery blood count information were included. NLR was computed as the ratio of the absolute neutrophil count to the absolute lymphocyte count, PLR was obtained by dividing the absolute platelet count by the absolute lymphocyte count, SII was calculated as platelet count × neutrophil count/lymphocyte count while lymphocyte-to-monocyte ratio (LMR)

was calculated as the ratio lymphocyte/monocyte. Information on blood counts were collected within a week before surgery. Curves of relapse free survival (RFS) were calculated using the Kaplan-Meier method. FFPE samples of the surgical specimen were collected to perform an RNAsequencing and subsequent analysis by CIBERSORT to assess the amount and composition of tumor-infiltrating immune cells.

Results: Forty-six patients were included in this single-center retrospective study, including 11 WDL, 15 DDL, 8 MFS, 12 UPS. No differences were seen in terms of RFS according to age or sex of the patients. As expected patients with G1 disease had a better RFS compared to G2 and G3 (110 VS 66 VS 56 months, P = 0,0494) and also histologies showed a significant difference in terms of RFS (WDL 110mo VS 79mo MFS Vs 56 mo DDL VS 53 mo UPS; P = 0,0234). Patients also had better RFS when SSI < 991 (P < 0,0001) NLR < 2,5 (P = 0,0001), PLR < 190 (P < 0,0001), LMR ≥2,4 (P = 0,0038).[Figure 1]
Tumor infiltrating cells fraction are shown in Figure 2. The correlation between LMR and intratumoral M2 macrophages did not reach statistical significance (p 0.352) however a trend of increase in M2 macrophages was seen as the LMR decreased.
Interestingly, as shown in Figure 3, patients with naive B-cell fraction (nBC) >3.13% had worse RFS compared to patients with B-cells ≤3.13% of the total tumor-infiltrating immune cell population (P = 0.0181). Furthermore, patients with activated dendritic cells (aDC) >1.02% had better RFS (P = 0.0222).
No intratumoral immune population reached a statistically significant correlation with the grading, however, as shown in figure 4, patients with higher histological grading seem to have a higher presence of intratumoral monocytes and M2 macrophages.

Conclusion: Our study confirms the prognostic role of intratumoral immune cells and systemic inflammatory markers.
Unlike what was shown in previous studies, the presence of high levels of activated B cells did not show a significant difference in terms of RFS while patients with high percentages of intratumoral nBC showed a worse RFS. Even more interestingly, in our study, patients with higher percentage levels of intratumoral aDCs showed better RFS, underlining the role of dendritic cells in the antitumor response.
The activity of intratumoral dendritic cells and the role of nBCs need to be investigated in subsequent studies with larger case series.
Likewise, the correlation between grading, monocytes and M2macrophages and between these and the LMR will be evaluated on a larger series to to strengthen its statistical validity.



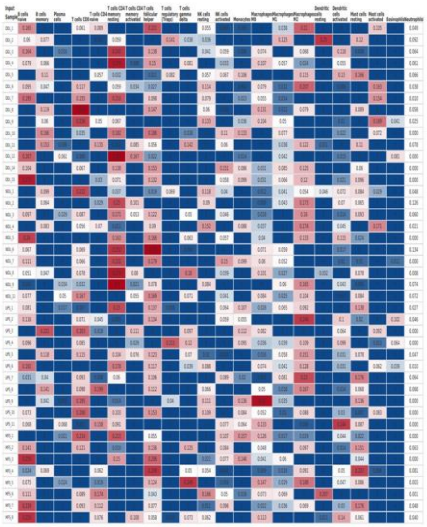


Figure 2. Intraumoral immune cells fractions according to CIBERSORT analysis

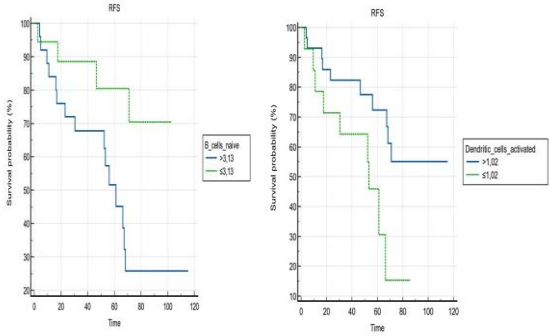


Figure 3. RFS according to intratumoral B cells and activated dendritic cells percentage

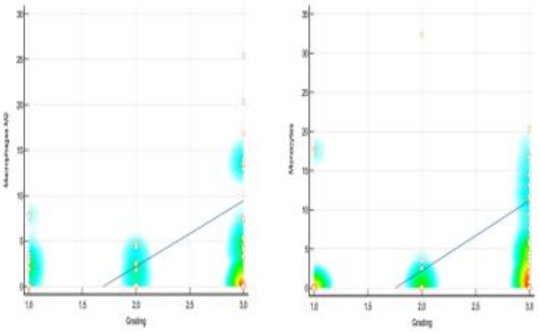


Figure 4. Correlation between M2 macrofages or Monocytes and grading

(P 365) MULTIMODAL ANALYSIS OF NUCLEAR AND GENOMIC ORGANIZATION IN RHABDOMYOSARCOMA AND NORMAL MYOGENIC CELLS

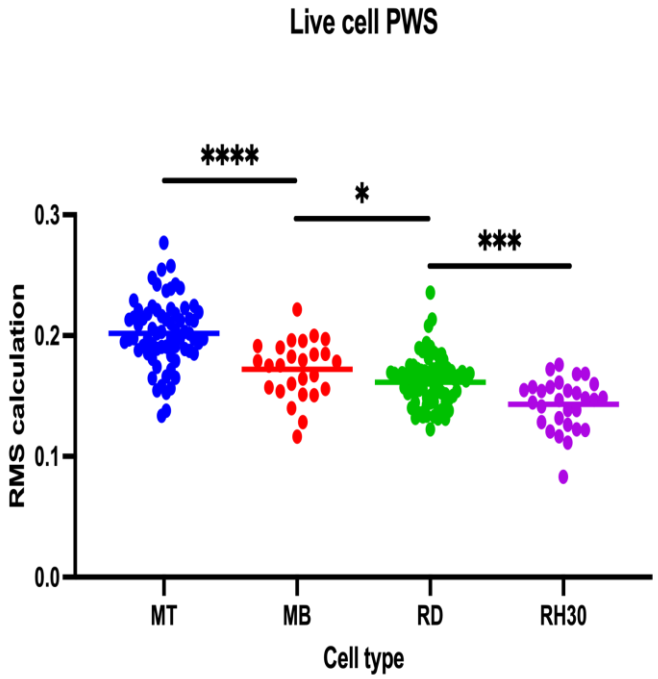
Kyle L. MacQuarrie, MD/PhD[1]; Tyler Hershenhouse, BS[2]; Joe Ibarra, BS[2]; Andrew Skol, PhD[2]; Luay Almassalha, MD/PhD[3], (1) Northwestern University Feinberg School of Medicine/Ann & Robert H Lurie Children's Hospital of Chicago, Chicago, IL, (2) Ann & Robert H Lurie Children's Hospital of Chicago, Chicago, IL, (3) Northwestern University Feinberg School of Medicine, Chicago, IL

Objective: Rhabdomyosarcoma (RMS), the most common soft-tissue pediatric sarcoma, is a tumor of skeletal muscle. A specific PAX-FOXO gene fusion is present in a subset of RMS tumors. The gene fusion status of RMS has significant effects on cellular biology and affects clinical outcomes. Myogenesis is the process by which proliferative myoblasts terminally differentiate into myotubes. As a tumor of skeletal muscle, RMS has been suggested to represent an arrested state of development between myoblast and myotube, resulting in continued proliferation.
Though RMS and normal myogenic cells share many similarities, such as gene expression and genome-wide binding patterns of myogenic regulatory factors, similarities and differences in higher-order chromatin structure and nuclear organization are less understood. Partial wave spectroscopic (PWS) microscopy can provide a quantitative assessment of chromatin heterogeneity in both individual and populations of live cells. We have recently shown myogenesis-dependent changes that are absent in RMS cells of the organization of specific chromosomes using fluorescent in situ hybridization.

Methods: To compare higher-order chromatin organization and nuclear organization within normal and RMS cells, normal myocytes and cell culture models of RMS were used. Normal proliferating myoblasts and terminally differentiated myotubes were compared to both PAX-FOXO fusion positive and negative RMS cell lines: fusion negative RD and fusion positive RH30 cells. Cells were imaged on glass bottomed dishes imprinted with a cell location grid enabling the tracking of specific nuclei throughout the imaging process. After live-cell PWS quantification of specific nuclei, cells were fixed, and fluorescence in-situ hybridization (FISH) for chromosomes 2 and 18 was performed. In addition, ATAC-Seq, RNA-Seq and Hi-C was performed in these cell types to characterize DNA accessibility, gene expression and DNA-DNA interactions, respectively.

Results: Initial analysis of population-level live-cell PWS data demonstrated statistically significant differences between all four cell types, with myotubes exhibiting the highest degree of heterogeneity, followed by myoblasts, then RDs, and finally RH30s (Figure 1). Imaging after FISH was performed has demonstrated that we can visualize specific chromosomes in individual cells of all cell types and analyze chromosomal organization data in the context of cell-specific PWS measurements.

Conclusion: Our initial analysis demonstrates that the terminally differentiated normal myotubes exhibit greater measures of chromatin heterogeneity compared to the proliferative myoblasts on the population level, potentially representing the significant changes in gene expression that accompany the morphological and cell cycle changes seen with differentiation. Interestingly, despite the known chromosomal complexity of the RMS tumor cell lines, they actually exhibited a lower measured level of heterogeneity compared to normal cells, with the fusion-positive tumor cells exhibiting the lowest level of all analyzed cell types. Further work will relate next-generation sequencing results with the imaging analysis of these cells.



(P 366) SURVIVAL DISPARITIES IN HISPANIC PATIENTS WITH SARCOMA TESTED WITH NEXT-GENERATION SEQUENCING (NGS)

Brandon E. Rose, MD, MPH[1]; Priscila Barreto coelho, MD[2]; Steven Bialick, DO[3]; Pooja Patel[4]; Kurt Statz-Geary, n/a[4]; Osvaldo Nunez, Jr.[4]; Alina Kang[4]; Mason Thornton[4]; Aditi Dhir, MD[4]; Emily Jonczak, MD[5]; Gina Z. D'Amato, MD[6]; Jonathan Trent, MD[5], (1) University of Miami and Jackson Health Systems, Miami Beach, FL, (2) University of Miami, Miami, FL, (3) Sylvester Comprehensive Cancer Center, University of Miami, Deerfield Beach, FL, (4) University of Miami Miller School of Medicine, Miami, FL, (5) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL, (6) Sylvester Comprehensive Cancer Center, Miami, FL

Objective: The objective of this study is to investigate whether there are survival disparities among Hispanic patients with sarcoma with next-generation sequencing (NGS) results, treated at a single institution. A prior study from our group in 2019 identified potential disparities in targetable mutations identified by NGS, which led to additional data collection on outcomes and with increased patient numbers. Given the unique demographics of our catchment area in South Florida, our large cohort of Hispanic patients allows us to perform more robust analyses and stratify by additional factors such as grade, stage, and histologic sarcoma subtype.

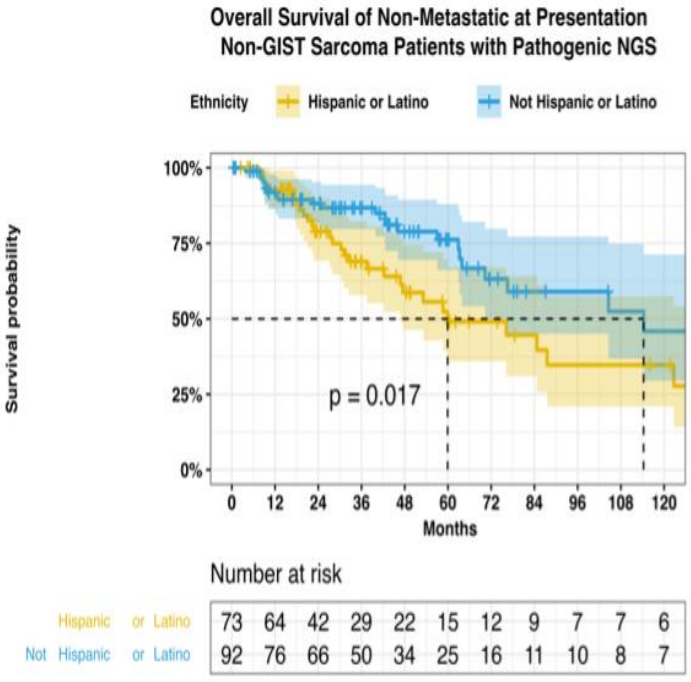
Methods: A retrospective analysis was performed on 541 patients with both non-GIST soft tissue and bone sarcoma with pathogenic variants found by NGS. We pooled 28,380 patient candidate medical record numbers using ICD10 codes, provider encounters, and past research projects, and queried our proprietary, institutional database, cancerSCOUT, for the pathogenic variants in NGS results from Caris, FoundationOne, and NeoGenomics. We obtained 1,433 unique NGS reports, ultimately yielding 589 sarcoma NGS reports with pathogenic variants

belonging to 541 patients after excluding non-sarcoma and GIST diagnoses. We systematically reviewed the clinical data available in the electronic health records to collect information related to diagnosis, survival, and treatment. Survival analysis was performed using Kaplan-Meier curves and Cox proportional hazards models. Demographic, clinical variables, and genetic alterations identified through NGS were assessed for their association with survival disparities in patients with sarcoma. Mutations were assigned as targetable based on the oncoKB version 4.5 with levels 1 to 4.

Results: Preliminary analysis of the collected data revealed significant survival disparities among Hispanic patients with pathogenic variants within NGS results. Hispanic patients who were non-metastatic at diagnosis had a statistically significantly worse median overall survival (mOS) of 59.9 months vs 114.3 months (p=0.017) compared to non-Hispanic patients. There were no significant differences in survival by ethnicity in this group of patients. Among the entire cohort, both with metastatic and localized disease, Hispanic patients were more likely to have targetable mutations via oncoKB levels 1 to 4. Interestingly, Hispanic patients were more likely to have targetable mutations at 66.5% compared to non-Hispanic patients at 52.6% (p = 0.012). The mutations that varied by ethnicity were as follows: TP53 54% of Hispanic NGS results vs 46.3% of Non-Hispanic NGS results; CDKN2A 12.4% of Hispanic NGS results vs 8.1% of Non-Hispanic NGS results; and PTEN 7.1% of Hispanic NGS results vs 3.0% of Non-Hispanic NGS results. Additional data is being analyzed to determine if differences in NGS results or with subsequent targeted treatment account for any of the noted survival differences. These additional analyses will be presented at CTOS.

Conclusion: These findings highlight the importance of considering both genetic and non-genetic factors in understanding and addressing survival disparities in Hispanic sarcoma patients. Our study reveals the survival disparities observed among Hispanic sarcoma patients with pathogenic NGS results. The integration of genetic information obtained through NGS with clinical and demographic variables allows for a more comprehensive understanding of the factors influencing patient outcomes. Further investigation is warranted to validate and expand upon these initial findings, with the goal of improving survival outcomes and reducing disparities in this patient population.

	Hispanic or Latino (N=170)	Not Hispanic or Latino (N=270)	Overall (N=440)
Birth Sex			
Female	105 (61.8%)	154 (57.0%)	259 (58.9%)
Male	65 (38.2%)	116 (43.0%)	181 (41.1%)
Stage at Diagnosis			
local	64 (37.6%)	83 (30.7%)	147 (33.4%)
locally advanced	15 (8.8%)	16 (5.9%)	31 (7.0%)
metastatic	33 (19.4%)	67 (24.8%)	100 (22.7%)
Unknown	2 (1.2%)	2 (0.7%)	4 (0.9%)
Grade of the sarcoma			
High	75 (44.1%)	127 (47.0%)	202 (45.9%)
Intermediate	41 (24.1%)	62 (23.0%)	103 (23.4%)
Low	4 (2.4%)	15 (5.6%)	19 (4.3%)
Unknown	10 (5.9%)	11 (4.1%)	21 (4.8%)
Age at diagnosis (years)			
Mean (SD)	50.9 (16.7)	53.4 (17.8)	52.4 (17.4)
Median [Min, Max]	51.0 [13.0, 89.0]	57.0 [12.0, 88.0]	54.0 [12.0, 89.0]
Received Systemic Treatment?			
Yes	130 (76.5%)	197 (73.0%)	327 (74.3%)
No	2 (1.2%)	6 (2.2%)	8 (1.8%)
Received Surgery?			
Yes	92 (54.1%)	169 (62.6%)	261 (59.3%)
No	7 (4.1%)	12 (4.4%)	19 (4.3%)
Received Radiation?			
Yes	66 (38.8%)	111 (41.1%)	177 (40.2%)
No	12 (7.1%)	33 (12.2%)	45 (10.2%)



(P 367) CHARACTERIZING HYPOXIA AND ASSOCIATED MOLECULAR FEATURES IN EXTREMITY SOFT TISSUE SARCOMA

Jen Dorsey*, PhD[1]; Joanna Przybyl, PhD[2]; Philippe Jolivet, BSc[2]; Peter Ferguson, MSc., MD[3]; Kim Tsoi, MD, PhD[3]; Jay Wunder, M.Sc, MD[3]; Peter Chung, MBChB MRCP FRCR FRCPC[4]; Edward Taylor, MSc, PhD[5]; Patrick Veit-Haibach, MD[6]; Elizabeth Demicco, MD, PhD[7]; Rebecca Gladdy, MD, PhD[8]; David Shultz, N/A, MD[4], (1) Lunenfeld Tanenbaum Research Institute/Mount Sinai Hospital, Toronto, ON, Canada, (2) McGill University, Montreal, PQ, Canada, (3) University of Toronto, Toronto, ON, Canada, (4) Princess Margaret Cancer Centre, Toronto, ON, Canada, (5) University Health network, Toronto, ON, Canada, (6) University Health Network, Toronto, ON, Canada, (7) Sinai Health System, Toronto, ON, Canada, (8) Mount Sinai Hospital, Sinai Health System, and Princess Margaret Cancer Centre Toronto, ON, Canada Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada University of Toronto, Toronto, ON, Toronto, ON, Canada

Objective: Undifferentiated pleomorphic sarcoma (UPS) and myxofibrosarcoma (MFS) are common extremity soft tissue sarcomas (STS) in adults with an approximate 50% risk of distant failure. Thus, there is a need to better understand the mechanisms of metastasis to increase therapeutic options and eventually improve outcomes. One known factor that correlates to aggressive STS disease is tumor hypoxia. We conducted a study to determine if hypoxia was detectable in extremity STS using exogenous hypoxia markers ([18F] fluoroazomycin-arabinoside (FAZA) and pimonidazole) and to evaluate associated epigenetic and expression changes.

Methods: We conducted a feasibility clinical trial (NCT03418818) to measure hypoxia in extremity or truncal STS with FAZA-PET/MRI. Patients in Arm A underwent a FAZA-PET/MRI prior to neoadjuvant radiation (RT) and patients in Arm B underwent a FAZA-PET/MRI prior to surgery and received adjuvant RT. We preferentially enrolled patients with UPS and MFS, however, other STS types were included. Prior to surgery, patients in Arm B received pimonidazole, and after tumor resection tissue was collected for correlative studies. Viable tumor regions were stained for pimonidazole and known endogenous hypoxia markers HIF-1α and CA-IX. To evaluate molecular changes due to hypoxia, we sorted dissociated tumor cells into pimonidazole + (hypoxic) and pimonidazole - (normoxic) populations by fluroescence activated cell sorting flow cytometry. Next, we performed DNA methylation analysis on hypoxic and normoxic clinical specimens (n=4) and on primary cell lines (UPS (n= 4) and MFS (n= 5)) exposed to hypoxic (0.1% O2) or normoxic conditions for 72 hours using EPIC microarrays (Illumina). We also performed bulk RNA sequencing of the same primary cell lines (Illumina). We conducted exploratory analyses of DNA methylation and RNA sequencing data using unsupervised clustering and principal component analysis. For differential methylation analysis we employed the ChAMP package, and for differential expression analysis we used the DESeq2 package in R. To identify significantly over- or underrepresented pathways in the gene expression data, we performed Gene Set Enrichment Analysis (GSEA).

Results: Twenty-five patients were enrolled (Arm A (n=15), Arm B (n=10)), the majority of whom had UPS (n=9) or MFS (n=10). Of the patients who had a successful baseline FAZA-PET/MRI, 14/20 patients demonstrated quantitative evidence of hypoxia, which correlated inversely with disease-free survival ($p < 0.05$, log rank test). Pimonidazole staining was heterogenous and colocalized with HIF-1α and CA-IX. We sorted dissociated tumor cells from six patients, but only four had enough DNA to proceed with the methylation analysis. Unsupervised and supervised analyses of DNA methylation data did not show any significant

changes associated with hypoxia in patient samples or cell lines. GSEA of RNA sequencing data identified significant enrichment of 102 genes involved in hypoxia and 54 genes involved in glycolysis in cell lines cultured in hypoxic conditions. Differential expression analysis identified 99 upregulated and 20 downregulated genes in cell lines cultured in hypoxic conditions compared to normoxic conditions (adjusted p value < 0.05). Ranked GSEA performed on the differentially expressed genes pointed to significant enrichment of genes activated by the HIF1α pathway in the cell lines cultured in hypoxic conditions, including CA9, FOS, NDRG1, LEP and EGLN3.

Conclusion: Overall, hypoxia was detectable in extremity STS using FAZA-PET/MRI and associated with worse disease-free survival. However, hypoxia was not associated with any DNA methylation changes in STS under the conditions evaluated. Future efforts will aim to identify hypoxia-specific expression alterations in UPS and MFS in this clinical cohort.

(P 368) DECODING THE IMMUNOGENOMIC DETERMINANTS OF RADIOTHERAPY RESPONSE IN SOFT TISSUE SARCOMAS

Siddh van Oost[1]; Debora Meijer, MSc[2]; Marieke Ijsselsteijn, PhD[1]; Brendy E. van den Akker, N/A, n/a[1]; Hans Baelde, n/a[1]; Inge H. Briaire-de Bruijn, N/A, n/a[1]; Anouk A. Kruiswijk, Drs.[3]; Michiel van de Sande, MD, PhD[4]; Pauline Wijers-Koster, n/a[1]; Rick Haas, MD, PhD[5]; Lukas Hawinkels, PhD[1]; Noel de Miranda, n/a[1]; Judith Bovee[1], (1) LUMC, Leiden, Zuid-Holland, Netherlands, (2) LUMC, Leiden, Noord-Holland, Netherlands, (3) Leiden University Medical Center, Amsterdam, Noord-Holland, Netherlands, (4) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (5) The Netherlands Cancer Institute, Amsterdam, Drenthe, Netherlands

Objective: Myxofibrosarcoma and undifferentiated soft tissue sarcoma (USTS) are two subtypes of genetically complex sarcomas with distinct morphological features. The mainstay of treatment is surgery, potentially in combination with neoadjuvant chemo- or radiotherapy. In recent years, patients with USTS or myxofibrosarcomas have experienced benefit from T cell immune checkpoint blockade that produced responses in about 25% of patients, mainly in USTSs. To improve our understanding of the immunobiology of these soft tissue sarcomas, we aimed to characterize the immune microenvironment through transcriptomic and immunophenotypic profiling.

Methods: In our retrospective study, we included 29 patients with high grade tumors (13 myxofibrosarcomas and 16 USTSs). Immune cell phenotypes and spatial interactions were mapped with a 40-marker imaging mass cytometry panel on tissues including pre-treatment biopsies, treatment-naïve resections and post-radiation resection. In parallel, immune-related gene signatures were obtained for treatment-naïve samples that underwent RNA sequencing, including seven myxofibrosarcomas and 13 USTSs. Histological response to radiotherapy was assessed by a bone and soft tissue pathologist and clinical follow-up data was collected for survival analysis.

Results: In comparison with other genetically complex sarcomas, both USTSs and myxofibrosarcomas presented an enrichment for hallmarks of ongoing inflammatory responses, as assessed through RNA sequencing analysis of immune-related genes. The immunologic

constant of rejection signature in combination with the microenvironment cell population counter deconvolution algorithm revealed a subgroup of highly infiltrated soft tissue sarcomas as well as a general enrichment for T cell-related signaling. The 33 immunophenotypes discovered with imaging mass cytometry provided important additional information regarding the actual levels of infiltration. In general, USTSs comprise higher densities of immune cells than myxofibrosarcomas, which might explain the reported differences in response to checkpoint blockade between both subtypes. Moreover, myxofibrosarcomas appeared to be relatively enriched for lymphoid immune cells like innate lymphoid cells and T cells, whereas USTS were more enriched for myeloid immune cells such as macrophages and monocytes. Intriguingly, we observed that both subtypes respond differently to radiotherapy, specifically that USTSs appear more radiosensitive than myxofibrosarcomas. By comparing the treatment-naïve microenvironment of responders with non-responders, we found that various types of T cells, as well as B cells and dendritic cells were more abundant in patients who responded to radiotherapy before treatment. These findings suggest a link between pre-existing anti-tumor immunity and responsiveness to radiotherapy.

Conclusion: In conclusion, high grade myxofibrosarcoma and USTS are generally considered immunogenic as assessed by both transcriptomic and immunophenotypic profiling. USTSs are more often immunogenic than myxofibrosarcomas and appear more sensitive to radiotherapy. Enrichment for pro-inflammatory hallmarks before treatment could potentially be used as a predictive biomarker for response to radiotherapy in USTS and for guiding combination treatment strategies.

(P 369) MAGEA ANTIGENS AS IMMUNOTHERAPEUTIC TARGETS FOR ADVANCED OSTEOSARCOMA

Debora Meijer, MSc[1]; Siddh van Oost[2]; Jessica Roelands, n/a[2]; Dina Ruano, PhD[2]; Inge H. Briaire-de Bruijn, N/A, n/a[2]; Brendy E. van den Akker, N/A, n/a[2]; Alwine Kruisselbrink, n/a[2]; Pauline Wijers-Koster, n/a[2]; Suk Wai Lam[2]; Michiel van de Sande, MD, PhD[3]; Hans Gelderblom, MD, PhD[3]; Marieke L. Kuijer, PhD[4]; Noel de Miranda, n/a[2]; Judith Bovee[2], (1) LUMC, Leiden, Noord-Holland, Netherlands, (2) LUMC, Leiden, Zuid-Holland, Netherlands, (3) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (4) Centre for Molecular Medicine Norway (NCMM), University of Oslo Leiden University Medical Center (LUMC), Leiden University, Oslo, Oslo, Norway

Objective: Osteosarcoma is the most common primary bone sarcoma. About 50% of patients develop metastatic disease and their 5-year survival lingers at around 20-30%. T cell checkpoint blockade immunotherapies have revolutionized cancer treatment in the last decade, but their impact remains limited in osteosarcoma. Therefore, we aimed to identify novel immunotherapeutic targets for osteosarcoma patients with advanced disease by exploring the immunological changes that accompany osteosarcoma progression.

Methods: We performed immunogenic characterization of a unique sample set containing multiple osteosarcoma samples of seven patients, collected during the course of disease. These included primary biopsies (n=5), primary resections (n=6), local recurrences (n=3) and metastases (n=10). For these samples, we performed RNA-sequencing and imaging mass cytometry to reveal the immunological landscape during osteosarcoma progression.

Results: Gene expression analysis of osteosarcoma samples revealed an increased cytotoxic immune signature in osteosarcoma metastases compared to primary tumors, which may result from a coordinated T cell driven anti-tumor immune response. Immunophenotyping with imaging mass cytometry further confirmed the increased T cell infiltrate in metastatic lesions. In parallel, we found a pronounced increase in the expression of cancer testis antigens in osteosarcoma metastases, in particular MAGEA-related antigens. Their overexpression in metastatic lesions was confirmed at protein level and, importantly, we demonstrated the presentation of MAGE-derived peptides in three osteosarcoma cell lines.

Conclusion: We provide evidence for a concomitant increase in cytotoxic anti-tumor immune responses and expression of MAGEA antigens from primary to metastatic osteosarcoma. This observation warrants the exploration of MAGEA antigens as immunotherapeutic targets for the treatment of advanced osteosarcoma.

(P 370) SYNOVIAL SARCOMA; ROLE OF ADJUVANT CHEMOTHERAPY IN SURGICALLY TREATED PATIETNS WITH NO METASTASIS; A SEER STAT ANALYSIS.

Mohamed Kelany, MBBCh, MD[1]; Amr Aly, MBBCh[2]; Ahmed Eldesoky, MBBCh, MD[3]; Asmaa.A. Abdeltawab, MBBCh, MD[4], (1) Faculty of medicines Ain Shams University, Cairo, Al Qahirah, Egypt, (2) Faculty of medicines Suez Canal University, Ismailia, Al Isma'iliyah, Egypt, (3) Faculty of medicines Mansoura University, Al Mansoura, Ad Daqahliyah, Egypt, (4) Faculty of medicines Asyut University, Asyut, Asyut, Egypt

Objective: Synovial sarcoma is known to be a unique subset of soft tissue sarcomas. Most types of sarcomas are chemo-resistant. However, regarding synovial sarcoma, there has been a debate whether it is considered chemo-sensitive or not. So, we aim to assess survival outcomes of adjuvant chemotherapy in surgically treated patients with non-metastatic synovial sarcoma.

Methods: In this retrospective cohort study, we extracted the data of 815 patients from the Surveillance, Epidemiology, and End Results (SEER) database whom were diagnosed between 2000-2019. All of them had Synovial Sarcoma with M0 disease. Stages I, II and III are considered in nodal negative patients. The diagnosis of stage IV synovial sarcoma is either based on N1 disease or M1 disease, so in this study we only included Surgically treated N1 Stage IV synovial sarcoma. The classification of tumor staging, nodal staging and metastasis were according to eighth edition of AJCC. We calculated the 5-year relative survival for patients who had adjuvant chemotherapy versus patients with no systemic therapy. Kaplan-Meier curve and log rank test were performed using SPSS 25 for survival analysis.

Results: The overall 5-year survival was 78.6%; P< 0.001. The 5-year relative survival of adjuvant chemotherapy(n=383) versus no systemic therapy(n=432) were 72% and 84.9% respectively; P< 0.001. With stratification by staging, the overall 5-year survival of adjuvant chemotherapy versus no systemic therapy were Stage I (76.2%, 90.4%; P< 0.001), Stage II (69.3%, 87.3%; P< 0.001), Stage III (63.4%, 67.5%; P< 0.001) and Stage IV (71.9%, 25.7%; P< 0.001. The median overall survival (OS) was 126 months in adjuvant arm and 152 months in patients with no systemic therapy.

Conclusion: Synovial Sarcoma has an overall favorable outcome. Adjuvant chemotherapy proved an overall no survival benefit compared to no systemic therapy. With stratification staging, Nodal negative Stages I, II and III patients showed better outcomes with no adjuvant chemotherapy. However, stage IV with Nodal metastasis benefited from adjuvant chemotherapy by 3 folds compared to no systemic therapy. In conclusion, stage IV synovial sarcoma are usually not indicated for surgery but if surgery was recommended for a selected patient, we recommend adjuvant chemotherapy only in nodal positive stage IV synovial sarcoma.

(P 371) DISRUPTION OF THE TP53 LOCUS IN OSTEOSARCOMA LEADS TO TP53 PROMOTER GENE FUSIONS AND RESTORATION OF PARTS OF THE TP53 SIGNALLING PATHWAY

Karim H. Saba, PhD[1]; Valeria Difilippo, Master[1]; Michal Kovac, PhD[2]; Louise Cornmark, PhD[1]; Linda Magnusson, N/A, MSc[1]; Jenny Nilsson, N/A, BSc[1]; Hilda van den Bos, PhD[3]; Diana C. J. Spierings, PhD[3]; Mahtab Bidgoli, BSc[4]; Tord Jonson, PhD[4]; Vaiyapuri Sumathi, MD[5]; Otte Brosjö, N/A, MD[6]; Johan Staaf, PhD[1]; Floris Foijer, PhD[3]; Emelie Styring, MD, PhD[4]; Michaela Nathrath, N/A, MD[7]; Daniel Baumhoer, MD[8]; Karolin Hansén Nord, PhD[1], (1) Lund University, Lund, Skane Lan, Sweden, (2) Slovak University of Technology, Bratislava, Bratislava, Slovakia, (3) University Medical Centre Groningen, Groningen, Groningen, Netherlands, (4) Skåne University Hospital, Lund, Skane Lan, Sweden, (5) Royal Orthopaedic Hospital, Birmingham, England, United Kingdom, (6) Karolinska University Hospital, Stockholm, Stockholms Lan, Sweden, (7) Klinikum Kassel, Kassel, Hessen, Germany, (8) University Hospital Basel, Basel, Basel-Stadt, Switzerland

Objective: TP53 is the most frequently mutated gene in human cancer. This gene shows not only loss-of-function mutations, but also recurrent missense mutations with gain- or separation-of-function activity. We have studied the primary bone malignancy osteosarcoma, which harbours one of the most rearranged genomes of all cancers. This is odd since it primarily affects children and adolescents who have not lived the long life thought necessary to accumulate massive numbers of mutations. In osteosarcoma, TP53 is often disrupted by structural variants, and we hypothesize that such rearrangement of the TP53 locus leads to the induction of cancer-driving genes by gene fusion or promoter swapping events.

Methods: Whole-genome mate-pair sequencing technology (Nextera Mate Pair, Illumina) was used to detect structural alterations affecting TP53 in a discovery cohort of 36 chemotherapy-treated resection specimens and a validation cohort of 36 treatment-naïve diagnostic biopsies from paediatric and adult conventional osteosarcoma patients. Whole genome DNA copy number alterations were investigated in 120 osteosarcomas, including the aforementioned cases (Cytoscan HD arrays, Thermo Fischer Scientific). For 68 osteosarcomas, 13 control osteoblastomas and in vitro cell models, high-quality RNA was obtained, and global gene expression analysis was performed using RNA sequencing (TruSeq, Illumina). To monitor intratumor heterogeneity among individual cells, single cell low-pass whole genome sequencing (0.01x, 100 cells per tumour) was applied to osteosarcoma cells.

Results: We show that TP53 structural variants are early events that not only facilitate further chromosomal alterations, but also allow the TP53 promoter to upregulate genes erroneously

placed under its control in a fashion accentuated by ongoing DNA damage. Paradoxically, many of the induced genes are part of the TP53-associated transcriptome, suggesting a need to counterbalance loss of TP53 function through separation-of-function mutations via promoter swapping. This mechanism was preferentially seen in patients with a younger age of osteosarcoma onset. Interestingly, introduction of a TP53 promoter gene fusion into a TP53-/- cell line shifted the global gene expression pattern back towards that of wild type cells, in line with the above findings.

Conclusion: Our findings demonstrate how massive genome errors can functionally turn the promoter region of a tumour suppressor gene into a constitutively active oncogenic driver. More specifically, we demonstrate that TP53 is not only lost in carcinogenesis, but its promoter can be selected for during tumour evolution and paradoxically rescue parts of the wildtype TP53-signalling pathway. This suggests that while the classical tumour suppressor activites of TP53 are lost, certain parts of the TP53 signalling pathway that are necessary for cancer cell survival and proliferation are retained. In conclusion, TP53 structural variants in osteosarcoma likely function similarly to separation-of-function hotspot TP53 missense variants seen in adult carcinomas.

(P 372) COPY NUMBER GAIN OF MDM2 IN ROD-SHAPED CHROMOSOMES PROVIDES CLUES TO EARLY STAGES IN CIRCULARIZED GENE AMPLIFICATION IN LIPOSARCOMA

Saskia Sydow, MSc[1]; Paul Piccinelli, N/A, PhD[2]; Shamik Mitra, PhD[1]; Panagiotis Tsagkozis, MD, Ph.D[3]; Asle C. Hesla, MD, PhD[4]; Camila Bedeschi Rego De Mattos, MD[2]; Jan Köster, MD[1]; Linda Magnusson, N/A, MSc[1]; Jenny Nilsson, N/A, BSc[1]; Adam Ameur, PhD[5]; Fredrik Mertens, MD, PhD[1], (1) Lund University, Lund, Skane Lan, Sweden, (2) Skåne University Hospital, Lund, Skane Lan, Sweden, (3) Karolinska University Hospital, Stockholm, Stockholms Lan, Sweden, (4) Karolinska University Hospital, Sollentuna, Stockholms Lan, Sweden, (5) Uppsala University, Uppsala, Uppsala Lan, Sweden

Objective: Well-differentiated liposarcoma (WDLS) is the most common subgroup of liposarcoma. The main characteristics are ring and/or giant marker chromosomes (RGMCs) involving amplification of chromosome arm 12q, including MDM2 and, often, HMGA2 and CDK4. RGMCs constitute a form of gene amplification, a common phenomenon in cancer, resulting in overexpression of key oncogenes. The underlying mechanism of the formation and development of RGMCs is poorly understood. It has been suggested that the initial event in this process is chromothripsis, followed by circularization and subsequent rounds of circular recombination. The objective of this study was to assess the formation and development of RGMCs by investigating lipomatous tumors with low-level gain of 12q as a potential route between lipomas and WDLS. Further, we evaluated the validity of the suggested model involving chromothripsis as the initial event.

Methods: The study included 28 tumor samples from 21 patients with lipomatous tumors. Seven lipomatous tumors with continuous gain (26-110Mb) of 12q in rod-shaped non-RGMC structures were compared to 12 tumor samples with gain/amplification of 12q in ring chromosomes showing at least one uninterrupted contig of copy number gain (>10 Mb). Short-read (shortWGS) and long-read whole genome sequencing (longWGS), genomic arrays (SNP

array), and mRNA sequencing (RNA-seq) were performed on selected samples to compare the characteristics of the two groups.

Results: The analysis of DNA from lipomatous tumors with rod-shaped chromosomes showed that the mechanisms behind gain of extra copies of 12q material vary considerably. In the simplest scenario, copy number (CN) gain of 12q was achieved through intrachromsomal duplication, followed by a few additional changes. None of these cases displayed genetic instability, and there was no CN oscillation, strongly arguing against a chromothriptic origin. In contrast, two other rod-shaped cases displayed multiple CN levels and numerous CN transitions, similar to findings in tumors with ring chromosomes. However, CN gained segments in rod-shaped chromosomes were less complex than those in rings, and both rod-shaped and circularized amplicons shared several key features, including lack of interspersed deletions; thus, none of the cases fulfilled the criteria for chromothripsis. ShortWGS and longWGS data showed more frequent internal structural variants (SVs) in CN gained regions of chromosome 12 (Chr12) than in other co-amplified chromosomes, suggesting that gain of Chr12 preceded that of other involved chromosomes. The data suggest a stepwise process, starting with gain of parts of Chr12 after the S-phase, followed by, possibly circularized, cycles of BFB events. Then, the supernumerary derivative Chr12 formed a dicentric chromosome with supernumerary material from another chromosome. Hence, the amplicons in ring chromosomes in WDLS are formed through a stepwise procedure that is more complex than previously recognized. Cluster analysis of RNA-seq data revealed no clear separation of tumors with rod-shaped chromosomes and tumors with ring chromosomes. However, a gradual increase in expression from normal fat over conventional lipomas to lipomatous tumors with rod-shaped chromosomes and then to lipomatous tumors with ring chromosomes was observed for CDK4, HMGA2 and MDM2. The gradual overlapping expression levels between the tumor types support the existence of an intermediary tumor type.

Conclusion: The study provides insights into the early steps of RGMC formation and suggests a potential evolutionary link between lipomas and WDLS. The amplicons in WDLS appear to develop through a complex, multistep mechanism, involving shattering of supernumerary DNA sequences, followed by recombination with other chromosomes as rod-shaped structures, and further gene amplification as ring chromosomes.

(P 373) DEDIFFERENTIATED AND HIGH GRADE CHONDROSARCOMAS: HOW DOES ISOCITRATE DEHYDROGENASE STATUS APPRISE PROGNOSIS AND THERAPY?

Karen E. Schoedel, MD[1]; Tanya Heim, M.S.[2]; Anette Duensing, MD[3]; Ines Lohse, PhD[2]; Rebekah Belayneh, MD[1]; Sumail Bhogal, MD[1]; Anya Singh-Varma, BS[3]; Alexander Chang, BS[2]; Uma Chandran, PhD[2]; Daniel F. Marker, MD/PhD[1]; Benjamin Nacev, MD, PhD[4]; Heather Szabo-Rogers, PhD[5]; Laura Presutti, RN, BSN[6]; Kurt R. Weiss, MD[2], (1) University of Pittsburgh Medical Center, Pittsburgh, PA, (2) University of Pittsburgh, Pittsburgh, PA, (3) University of Pittsburgh School of Medicine, Pittsburgh, PA, (4) University of Pittsburgh Hillman Cancer Center, Pittsburgh, PA, (5) University of Saskatchewan, Saskatoon, SK, Canada, (6) Rocky Vista College of Medicine, Pittsburgh, PA

Objective: High grade and dedifferentiated chondrosarcomas (CSAs) are frequently associated with isocitrate dehydrogenase (IDH) mutations and often exhibit a poor clinical outcome.

Treatment is limited mainly to surgery. Defining IDH status {wild type (WT) and mutant} and the associated transcriptome may prove useful in determining other therapeutic options in these neoplasms.

Methods: Formalin fixed paraffin embedded material from 69 primary and recurrent grades 2, 3 and dedifferentiated (CSAs) was obtained. DNA sequencing for IDH1 and IDH2 mutations (n= 47) and RNA sequencing via Nextseq 2000 (n=14) were performed. Differentially expressed genes (DEGs) were identified and used to predict aberrant biological pathways with Ingenuity Pathway Analysis (IPA) software (Qiagen). Gene Set Enrichment Analyses (GSEA) using subsets C3, C5 and C7 were performed. Differentially expressed genes WT1, AR and SATB2 were validated by immunohistochemistry. Outcome analysis was performed using the Wilcoxon Test.

Results: A set of 69 (CSAs), 28 females, 41 males, average age 65, distributed among femur, pelvis, humerus, and chest wall were identified from available clinical material. After further selection based on available IDH status, we evaluated 15 IDH WT and 32 IDH mutant tumors as part of this dataset. 7 of 15 IDH WT tumors involved the chest wall/scapula, while 1 of 32 mutants arose in the scapula. There were far more genes overexpressed in IDH WT tumors compared to IDH mutant tumors (Fig. 1). Validation immunohistochemistry demonstrated expression of WT1 and AR in IDH WT tumors, but not in IDH mutants. SATB2 was expressed in IDH mutant tumors and not in WT tumors. Furthermore, IDH WT and IDH mutant tumors were transcriptomically distinct in the IPA and GSEA with IDH mutant tumors showing increased activity in methylation pathways and endochondral ossification, while IDH WT tumors showed more activity in normal matrix development pathways (data not shown). Outcome analysis revealed differences in overall survival between mutant and WT tumors (p=0.04), dedifferentiated mutant and high grade mutant tumors (p=0.03) and dedifferentiated mutant and high grade WT tumors (p=0.03) (Fig. 2). Longest survival times were observed in patients with high grade WT tumors while patients with dedifferentiated mutant tumors showed lowest survival. Generally, patients with IDH WT tumors displayed longer survival in both high grade and dedifferentiated groups.

Conclusion: High grade and dedifferentiated chondrosarcomas are further characterized by IDH status which in turn informs transcriptomic phenotype and overall survival. The transcriptome is distinct depending on IDH status and implies different treatment targets.

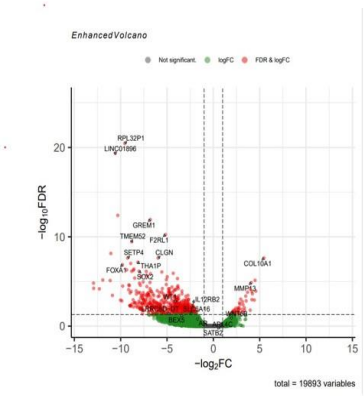


Fig. 1 Volcano plot depicting differences in gene expression between *IDH* WT (left side of graph) and *IDH* Mutant chondrosarcomas (right side).

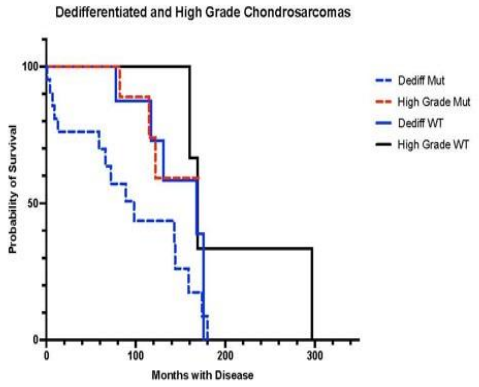


Fig. 2 Kaplan-Meier plot of *IDH* mutant (both *IDH1* and *IDH2*) vs. WT overall survival in dedifferentiated and high grade chondrosarcomas

(P 374) ARHGEF4 EXPRESSION IN SOFT TISSUE SARCOMA CORRELATES WITH LOCAL GROWTH PATTERN AND LOCAL RECURRENCE: COMPREHENSIVE GENOMIC ANALYSIS IN SEARCH OF BIOMARKER OF LOCAL GROWTH PATTERN

Yongsung Kim, Dr.[1]; kyoungbun lee, M.D. PhD.[2]; Han-soo Kim, N/A, MD. PhD.[3]; Shinn Kim, MD[2]; Jay Hoon Park, MD[2]; Jeonghwan Youk, MD. PhD.[2]; ha Jeong kim, PhD.[2]; Ilkyu Han, MD. PhD.[2], (1) Seoul National University Bundang Hospital, Gyunggi-do, Kyonggi-do, Republic of Korea, (2) Seoul National University Hospital, Seoul, Seoul-t'ukpyolsi, Republic of Korea, (3) Seoul National University Hospital, 101, Daehak-ro, Jongno-gu, Seoul-t'ukpyolsi, Republic of Korea

Objective: Soft tissue sarcoma(STS) is known to respect barriers to tumor spread while its infiltrative and expanding growth patterns are not investigated in molecular level. We hypothesized presence of genes up regulated or down regulated during local growth of STS. We have explored to discover candidate genes involved in the growth pattern of soft tissue sarcoma and sought its correlation with clinical outcomes including local recurrence and metastasis.

Methods: We have collected tumor cells from central and peripheral areas separately and performed Whole transcriptome sequencing(WTS) in 3 Undifferentiated Pleomorphic Sarcoma (UPS) and one pleomorphic liposarcoma (P-LPS) tissues. We have selected 58 candidate genes and performed real time PCR to choose the genes that showed higher expression in peripheral areas than central areas of STS. ARHGEF4, which is mainly located at cytoplasm and involved in regulation of actin cytoskeleton, showed increased expression in periphery than central tumor. To investigate the role of ARHGEF4 in cell invasion, migration, and cell viability, Boyden chamber assay, wound healing assay, and MTT assay were performed with patient-derived STS cell lines after knock down of ARHGEF4 by short interfering RNA(siRNA). We have performed immunohistochemistry for ARHGEF4 expression in biopsy specimens of 66 patients with UPS and myxofibrosarcoma(MFS).

Results: Knockdown of ARHGEF4 by siRNA resulted in decreased cell invasion, migration, and cell viability in patient-derived STS cell lines. Expression of ARHGEF4 showed correlation with infiltrative and expanding histologic growth pattern (p=0.023). The elevated expression of ARHGEF4 was associated with a trend towards decreased local recurrence-free survival (p=0.148).

Conclusion: These results suggest ARHGEF4 could serve as a biomarker and a therapeutic target of local growth pattern in STS.

(P 375) IDENTIFICATION OF METASTATIC CELLS IN UNDIFFERENTIATED PLEOMORPHIC SARCOMA: SINGLE CELL RNA SEQUENCING AND CIBERSORT ANALYSIS

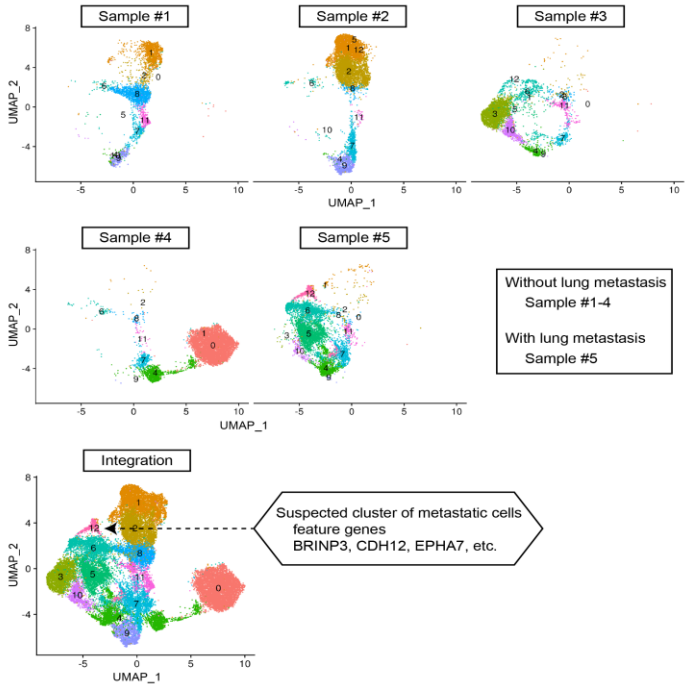
Eijiro Shimada, MD/PhD[1]; Joy Ban, PhD[2]; Vijitha Puviindran, BS[3]; Asjah L. Wallace, BS[1]; Makoto Nakagawa, MD, PhD[3]; Koji Ishikawa, MD, PhD[1]; Benjamin Alman, MD[3], (1) Duke University, Chapel Hill, NC, (2) Duke Univeristy, Durham, NC, (3) Duke University, Durham, NC

Objective: Undifferentiated Pleomorphic Sarcoma (UPS) is one of the most common types of malignant soft tissue sarcoma. It has a high recurrence rate of around 40% and a discouraging prognosis, with a 5-year survival rate of 50-60%. Lung metastasis accounts for 90% of distant spread in UPS, suggesting that preventing lung metastasis could improve patient outcomes. Research on tumor heterogeneity, the diverse characteristics of cells within a tumor, has been growing. In UPS metastasis, this heterogeneity also has received attention, and our research lab has contributed by discovering the coexistence of metastatic and non-metastatic cells in the same tumor. This study aims to identify and understand these elusive metastatic cells in UPS, enhancing our knowledge and potentially leading to innovative treatments.

Methods: We collected five samples of primary tumors diagnosed as UPS. Among the collected samples, one exhibited lung metastasis at the time of sampling. Single-cell RNA sequencing (scRNA-seq) was performed on the collected samples, and clustering was conducted after integration. We detected distinctive clusters associated with the samples exhibiting lung metastasis and identified feature genes specific to those clusters. Furthermore, we obtained bulk expression data of UPS from The Cancer Genome Atlas and analyzed the presence of clusters in the results of scRNA-seq for each sample using the analysis tool called CIBERSORT. By comparing tumors with lung metastasis to tumors without lung metastasis, we validated the distinctive clusters associated with tumors that had lung metastasis.

Results: After filtering the five samples based on sample quality, we were able to analyze 3777, 6629, 4664, 7074, and 6060 cells in each respective sample. Integration was performed based on canonical correlation analysis, followed by a shared nearest neighbor modularity optimization based clustering, resulting in the identification of 13 clusters. Among these clusters, three clusters (cluster #5, #6 and #12) were found to be more abundant in the samples from primary tumors with lung metastasis compared to the ones without lung metastasis. Furthermore, according to the results obtained from CIBERSORT analysis, cluster #12 was relatively abundant in tumors with lung metastasis. Analyzing the feature genes of cluster #12 based on these findings revealed an upregulation in the expression of genes such as BRINP3, CDH12, and EPHA7.

Conclusion: By conducting analysis using scRNA-seq and CIBERSORT, we were able to identify genes that characterize metastatic cells in UPS. The functional evaluation of these genes, both in vitro and in vivo, is a future task to determine how they contribute to metastatic ability.



(P 376) MULTI-OMIC PROFILING OF LEIOMYOSARCOMA IDENTIFIES MARKERS PREDICTIVE OF RESPONSE TO GEMCITABINE AND DOCETAXEL

Joanna Przybyl, PhD[1]; Pegah Rahimizadeh, MSc[1]; Philippe Jolivet, BSc[1]; Sujay Vennam, BSc[2]; Matt van de Rijn, M.D.[2]; Paul Huang, PhD[3], (1) McGill University, Montreal, PQ, Canada, (2) Stanford University, Stanford, CA, (3) The Institute of Cancer Research, London, England, United Kingdom

Objective: Combination of gemcitabine and docetaxel (Gem/Tax) is one of the most frequently considered systemic first-line regimens for leiomyosarcoma (LMS) patients with advanced disease. However, there is wide heterogeneity in clinical responses with only a subset of patients responding to this treatment. There is currently a poor understanding of the biology underlying this differential response and there are no predictive biomarkers for the response to Gem/Tax in LMS. The identification of biomarkers involved in the response to Gem/Tax would allow clinicians to treat only those patients that are most likely to receive long-term durable benefit, while sparing the toxicity of drug exposure for those patients who are unlikely to benefit this therapy.

Methods: We profiled archival tumor specimens from the Leiomyosarcoma Direct Research Foundation (LMSdr) Tumor Bank hosted at Stanford University. The LMSdr Tumor Bank

contains fully annotated 288 primary, recurrent and metastatic tumor specimens that were used to construct a tissue microarray. Among these cases, we identified a cohort of primary specimens from 54 patients who have been treated with Gem/Tax (26 responders and 28 non-responders). To identify candidate mechanisms of Gem/Tax resistance and response in LMS, we performed multi-omic profiling of these specimens by whole transcriptome RNA-seq (Illumina), DNA methylation profiling (EPIC microarrays, Illumina). High-throughput proteomic profiling, miRNA sequencing and validation experiments by immunohistochemistry are ongoing.

Results: Between responders and non-responders to Gem/Tax therapy, we identified 41 significantly differentially methylated CpGs, and 163 significantly differentially expressed genes. Differentially methylated CpGs were associated with 25 protein-coding genes. Among the differentially methylated CpGs, hypermethylation in the gene body of the RGL3 in two separate CpGs was positively correlated with mRNA expression of this gene ($r = 0.53$ and $p=0.005$ for cg17848054, and $r = 0.46$ and $p=0.02$ for cg02774542). Genes significantly upregulated in responders included the COL1A1 oncogene and several genes encoding protein kinases, transcription factors and growth factors. We validated strong expression of the COL1A1 protein in the primary tumors of responders to Gem/Tax by immunohistochemistry (strong expression in 20/25 (80%) of responders compared to 12/27 (44%) of non-responders; Fisher's exact test $p=0.01$). In the non-responders, the significantly upregulated genes included PAK3 protein kinase and THRB transcription factor. Gene Set Enrichment Analysis indicated higher expression of the genes involved in the IGF1R, PDGF and thrombopoietin signaling pathways in responders to Gem/Tax. Analysis of the paired primary and metastatic tumors from 3 patients identified 66 differentially expressed genes. Metastatic tumors demonstrated significant overexpression of the gene encoding oncogenic ROS1 protein kinase. By immunohistochemistry, we validated expression of ROS1 protein in 134/261 (51.3%) of all specimens included in the tissue microarray. Metastatic tumors showed higher expression of ROS1 compared to primary tumors (expression of ROS1 in 68% (39/57) of metastatic tumors, compared to 47% (95/204) of primary tumors; Fisher's exact test $p=0.004$). Among patients treated with Gem/Tax, 13/17 (76%) of metastatic tumors showed expression of ROS1 compared to 27/53 (51%) of primary tumors (Fisher's exact test $p=0.09$).

Conclusion: This is the first study to identify candidate markers and molecular pathways associated with response and resistance to Gem/Tax therapy in LMS. This is also the first study that demonstrates the expression of ROS1 protein kinase in LMS, which can be potentially therapeutically targeted by crizotinib. Our findings warrant further studies (including proteomics, miRNA profiling and immunohistochemistry) on the current cohort and in an independent cohort to validate these findings.

(P 377) EFFECTIVENESS OF INTERVENTIONAL RADIOLOGY GUIDED PROCEDURES FOR LOCALLY RECURRENT AND METASTATIC SARCOMA: SINGLE INSTITUTIONAL RESULTS

Gayathri Vijayakumar, BS[1]; Rahim Laiwalla, MS[2]; Austin Yu, BS[3]; Anel Yakupovich, MD[2]; Jordan Tasse, MD[2]; Alan T. Blank, MD, MS[2], (1) Rush University Medical Center, Edison, NJ, (2) Rush University Medical Center, Chicago, IL, (3) Rush University Medical Center, Alhambra, CA

Objective: Musculoskeletal image guided interventional radiology (IR) techniques have grown popular in the management of sarcoma. Thermal ablation and nonthermal ablative techniques are minimally invasive, targeted treatment modalities for sarcoma that can be seamlessly integrated into neoadjuvant or adjuvant treatment regimens. We report our institutional cohort of metastatic and recurrent sarcomas treated by IR.

Methods: From 2013 to 2022, 24 patients who received an ablation procedure for recurrent or metastatic sarcoma at our institution were retrospectively reviewed. Patient's sarcoma treatments including surgical resection, chemotherapy and radiotherapy, ablation modality, postprocedural complications, ablated lesion progression, and clinical course were collected from the medical records.

Results: Sixteen patients were treated with ablation for metastatic sarcoma and eight patients were treated with ablation for locally recurrent sarcoma. Median size of the ablated lesion was 2.7 cm (IQR 1.75-4.43). Lesion progression was observed in 2 of 24 ablated lesions at median 8 months, both in ablated locally recurrent sarcoma. Four patients developed complications including nerve pain, hemoptysis, infected hematoma, and necrotic wound requiring debridement.

Conclusion: We report high levels of local control of the ablated lesions in our cohort with minimal post-procedural complications. Future multi-institutional studies should evaluate the efficacy of ablation techniques in a more uniform cohort.

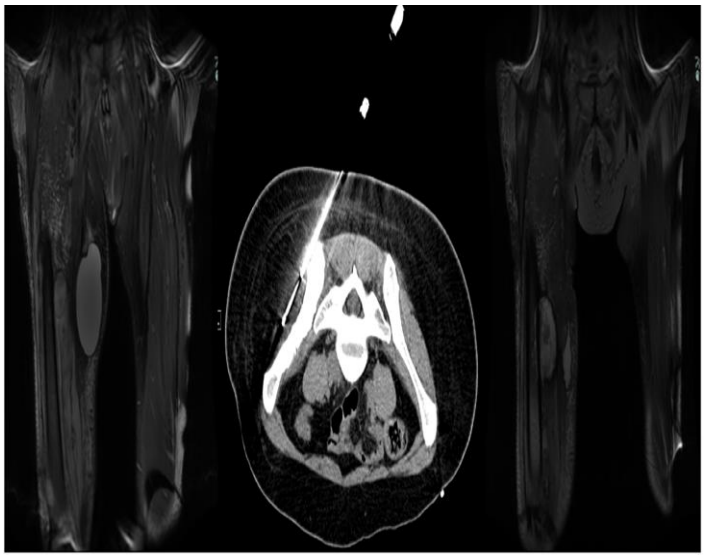


Figure 1: Pre-ablation Coronal T2 FS MRI demonstrating myxofibrosarcoma in left thigh (left). IR guided cryoablation of the lesion (middle). Post-ablation Coronal T2 FS MRI 10 months following cryoablation demonstrating stable lesion (right).

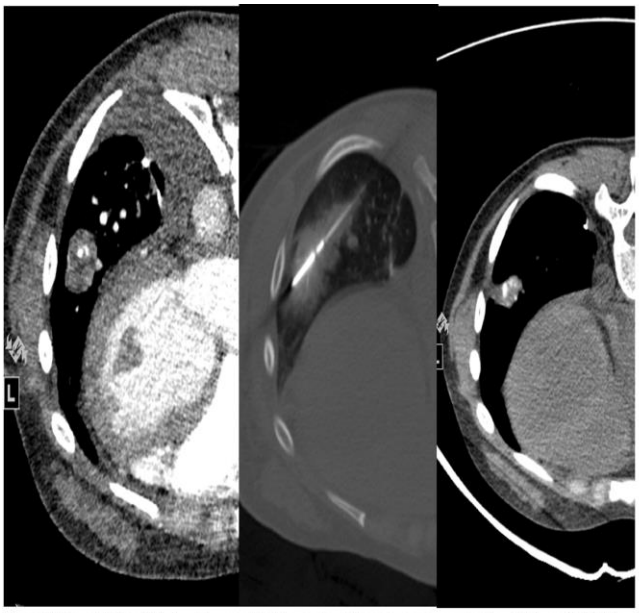


Figure 2: Pre-ablation CT chest demonstrating metastatic osteosarcoma to the left lung (left). IR guided cryoablation of the lesion (middle). Post-ablation CT chest at 6 months following cryoablation demonstrating decreased size of metastatic lesion.

(P 378) MULTIOMICS PROFILING OF EPITHELIOID SARCOMA UNVEILS EPIGENETIC CIRCUITRIES THAT UNDERLIE THE PATHOBIOLOGY OF PROXIMAL AND CLASSIC SUBTYPES: RESULTS FROM THE ISG EPISOBS OBSERVATIONAL STUDY

Luca Sigalotti, PhD[1]; Anna Maria Frezza, MD[2]; Marta Sbaraglia, MD[3]; Elisa Del Savio, PhD[4]; Davide Baldazzi, PhD[4]; Michele Doni, MSc[1]; Veronica Varano, MSc[1]; Chiara Pastrello, PhD[5]; Marco Gambarotti, MD[6]; Bruno Vincenzi, MD, PhD[7]; Antonella Brunello, MD, PhD[8]; Giacomo Giulio Baldi, MD[9]; Emanuela Palmerini, MD, PhD[10]; Beatrice Valenti, PhD[4]; Giulia Tedesco, MSc[1]; Ilaria De Benedictis, MSc[4]; Sandro Pasquali, MD, PhD[11]; Matilde Ingrosso, MSc[12]; Viviana Appolloni, PhD[13]; Marta Barisella, MD[14]; Nadine Narducci, MD[15]; Alessandro Gronchi, MD, FSSO[16]; Igor Jurisica, PhD[5]; Silvia Stacchiotti, MD[2]; Paolo Giovanni G. Casali, MD[17]; Angelo Paolo Dei Tos, N/A, MD[18]; Roberta Maestro, PhD[1], (1) Unit of Oncogenetics and Functional Oncogenomics, Centro di Riferimento Oncologico di Aviano IRCCS, Aviano, Italy, Aviano, Friuli-Venezia Giulia, Italy, (2) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (3) Department of Pathology, Azienda Ospedale-Università Padova, Padova, Italy, Padova, Veneto, Italy, (4) Unit of Oncogenetics and Functional Oncogenomics, Centro di Riferimento Oncologico di Aviano IRCCS, Aviano, Italy, Aviano (PN), Friuli-Venezia Giulia, Italy, (5) Data Science Discovery Centre for Chronic Diseases, Krembil Research Institute, Toronto Western Hospital, University Health Network, Toronto, Canada, Toronto, ON, Canada, (6) Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (7) Università Campus Biomedico di Roma, Roma, Italy, Rome, Lazio, Italy, (8) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy, Padua, Veneto, Italy, (9) Medical Oncology Department, "Santo Stefano" Hospital, Prato, Italy, Prato, Toscana, Italy, (10) IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (11)

Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (12) Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy, (13) Italian Sarcom Group, terni, Umbria, Italy, (14) Department of Pathology,Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (15) Department of Medicine, University of Padua School of Medicine, Padova, Italy, Padua, Veneto, Italy, (16) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (17) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (18) Department of Medicine, University of Padua School of Medicine, Padova, Italy, Padova, Veneto, Italy

Objective: Epithelioid sarcoma (ES) is an ultra-rare sarcoma hallmarked by loss of SMARCB1/INI1 expression. Based on cytomorphology, ES is distinguished into classic (C-ES) and proximal (P-ES) subtypes, irrespective of anatomic site. This distinction has clinical relevance, as P-ES is associated with a more aggressive behavior and poorer outcome. Whether this behavior is an intrinsic biological feature of P-ES or rather reflects its often proximal and deeper anatomic location remains to be understood. Apart from the SMARCB1/INI1 loss, little is known about the biology of ES and the determinants of the distinctive clinicopathologic features of the two subtypes. To shed light on this we performed a multiomics analysis of a cohort of 24 ES, in the framework of the Italian Sarcoma Group (ISG) observational study focused on ES EpisObs (NCT03099681).

Methods: Clinically annotated samples were collected from patients in the retrospective and prospective cohorts of the EpisObs ISG study, selecting primary, untreated ES, with available material suitable for molecular analyses. Diagnosis was confirmed through a centralized pathology review by expert pathologists, according to the 2020 WHO. FFPE sections with a tumor cell fraction ≥ 60% were selected for multiomics profiling, including targeted-DNA, RNA and miRNA NGS sequencing, and DNA methylation (methyl-DNA) profiling. NGS DNA, RNA, and miRNA libraries were run on Illumina platforms and Illumina Infinium MethylationEpic arrays were used for methyl-DNA analysis. Methyl-DNA:RNA and miRNA:RNA data were anticorrelated using Spearman correlation test. Selected differentially expressed genes were orthogonally validated by IHC. HSES2R, VAESBJ and Epi544 cell lines were used for in vitro miRNA validations and drug response.

Results: The study cohort was composed of 14 C-ES and 10 P-ES, all INI1 negative at IHC (Table 1). Apart from SMARCB1 deletion, no recurrent mutation events were detected. Conversely, P-ES and C-ES clearly differed for RNA, methyl-DNA, and miRNA profiles, forming distinct clusters in unsupervised analyses (Figure 1). Pro-aggressive transcriptional signatures were tendentially enriched in P-ES, which featured upregulation of genes involved in chromatin metabolism or cell cycle, and downregulation of genes associated with mesenchymal features. This was in line with methyl-DNA results that indicated that DNA methylation was higher in P-ES and mostly affected genes involved in cell migration and vasculogenesis. Functional annotation of the genes anticorrelated with the cognate miRNAs indicated that miRNAs also play an important role in shaping ES transcriptome, and miRNA and RNA data integration unveiled novel circuits contributing to the transcriptional features of the two subtypes. Among these, a complex circuit connected miR-137 and miR-24 with HELLS, EZH2, MYC, and E2F, and sustained cell proliferation and deregulation of chromatin metabolism in P-ES. In this circuit HELLS, which is regulated by both miRNAs and promotes DNA methylation-mediated gene silencing, acts as link between these two epigenetic mechanisms of transcriptional regulation. Finally, some differentially expressed genes whose expression may discriminate P-ES and C-ES were validated by IHC analysis.

Conclusion: This multiomics study supports the notion that P-ES and C-ES are distinct

molecular entities, characterized by a different degree of phenotypic drift form the mesenchymal makeup and marked by different DNA methylation and miRNA:gene circuitries. The transcriptional profile of P-ES suggests that biology rather than anatomic location sustains its poorer outcome. Our study unveiled also novel immunohistochemical markers that might aid in the differential diagnosis of P-ES and C-ES, thus serving as potential tools of patient stratification in future studies.

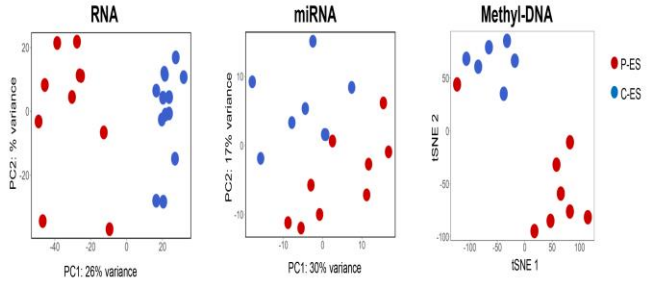
Table 1. Patients characteristics

	Gender	Age	ES subtype	Primary site	Stage at diagnosis	Primary treatment	Outcome	Status*
1	F	47	Proximal	Perineum	Localised, resectable	Surgery, post-operative CT and RT	Development of LR and DM	DOD
2	F	36	Proximal	Groin	Localised, resectable	Surgery	Development of DM	DOD
3	F	32	Proximal	Groin	Localised, resectable	Surgery	No recurrence	Alive, NED
4	F	34	Proximal	Vulva	Locally advanced, resectable	Surgery, pre- and post-operative CT	Development of DM	DOD
5	M	60	Proximal	Groin	Locally advanced, unresectable	CT	Development of DM	DOD
6	F	49	Proximal	Groin	Localised, resectable	Preoperative CT, surgery	No recurrence	Alive, NED
7	F	44	Proximal	Groin	Localised, resectable	Surgery	Development of LR and DM	AWD
8	F	45	Proximal	Trunk	Localised, resectable	Surgery	Development of LR	Alive, NED
9	F	70	Proximal	Gluteus	Localised, resectable	Surgery	Development of LR	Alive, NED
10	F	23	Proximal	Skull	Localised, resectable	CT, surgery	Development of DM	AWD
11	M	28	Classic	Axilla	Localised, resectable	Surgery, post-operative RT	Development of LR and DM	DOD
12	F	14	Classic	Forearm	Localised, resectable	Surgery	Development of LR and DM	DOD
13	F	20	Classic	Leg	Localised, resectable	Surgery, post-operative RT	Development of LR and DM	DOD
14	M	21	Classic	Buttock	Localised, resectable	Pre-operative RT, surgery	Development of LR	Alive, NED
15	M	16	Classic	Hand	Localised, resectable	Surgery	Development of LR and DM	Alive, NED
16	M	32	Classic	Trunk	Localised, resectable	Pre-operative RT and CT, surgery	Development of DM	DOD
17	M	35	Classic	Hand	Localised, resectable	Surgery	Development of LR	Alive, NED
18	M	26	Classic	Arm	Localised, resectable	Surgery, post-operative RT	Development of DM	DOD
19	M	28	Classic	Forearm	Localised, resectable	Surgery, post-operative RT	Development of DM	DOD
20	M	56	Classic	Forearm	Localised, resectable	Surgery	Development of LR	Lost to FU
21	F	21	Classic	Hand	Localised, resectable	Surgery	No evidence of disease	Alive, NED
22	F	32	Classic	Leg	Localised, resectable	Surgery	No evidence of disease	Alive, NED
23	M	55	Classic	Arm	Localised, resectable	Surgery (amputation), post-operative RT	No evidence of disease	Alive, NED
24	M	19	Classic	Foot	Locally advanced, resectable	Surgery (primary), CT, surgery (locoregional lymph-nodal metastases), RT	No evidence of disease	Alive, NED

* Status: follow up was updated in the last 6 months.

F: female; M: male; CT: chemotherapy; RT: radiation therapy; LR: local recurrence; DM: distant metastases; DOD: dead of disease; NED: no evidence of disease; AWD: alive with disease; FU: follow up.

Figure 1



(P 379) THE ROLE OF EZHIP IN DRIVING AGGRESSIVE OSTEOSARCOMA

Wajih Jawhar, PhD Student[1]; Geoffroy Danieau, PhD[1]; Alva Annett, PhD student[1]; Sungmi Jung, MD[2]; Robert Turcotte, MD[3]; Swneke Donovan Bailey, PhD[3]; Lingxin Zhang, MD[4]; Nada Jabado, MD, PhD[3]; Livia Garzia, PhD[1], (1) Research Institute of the McGill University Health Center, Montreal, PQ, Canada, (2) Department of Pathology, McGill University Health Center, Montreal, PQ, Canada, (3) McGill University, Montreal, PQ, Canada, (4) Mount Sinai Hospital/University of Toronto, Toronto, ON, Canada

Objective: Osteosarcoma (OS) is the most common bone cancer and accounts for 20% of all bone tumours. OS is a disease of genomic instability characterized by extensive structural variants and somatic copy number alteration that frequently lead to gain in oncogene expression and loss of tumor suppressors. While oncogenic mutations in histone proteins (oncohistones) have also been found in osteosarcoma tumors, very little is known about the role of epigenetics and chromatin remodeling in these malignancies. We hypothesized that histone mutations and aberrant expression of the onco-histone mimic, EZHIP, play a role in osteosarcoma pathogenesis.

Methods: The expression of oncohistones (H3K27M and H3G34W), and the oncohistone-mimic EZHIP was evaluated by immunohistochemistry in a cohort of 55 pediatric and adult osteosarcomas. We investigated the presence of co-occurring partner mutations by molecular profiling (WES and WGS) and clinical correlates with EZHIP expression.To study the role of EZHIP in OS pathogenesis, we used patient derived OS cell models of different genetic backgrounds. Using gain of function (lentiviral mediated overexpression) and loss of function modalities (CRISPR-Cas9) we investigated the oncogenic role of EZHIP and the consequences of its loss on the epigenome, transcriptome and on OS cells tumorigenic capacity in-vivo..

Results: EZHIP is highly expressed in a subset of osteosarcomas in both our discovery cohort and an external validation cohort of 28 additionalOS cases. EZHIP overexpression in the osteosarcoma cell of origin (human mesenchymal stroma cells) stalls its differentiation capacity into mature bone tissue and rather enhances its proclivity to differentiate towards the myogenic lineage and upregulates of oncogenic pathways. In high-grade OS cell lines, overexpression of EZHIP promotes tumorigenesis and enhances aggressiveness resulting in shorter survival time in orthotopically implanted mouse models. Similarly, the loss of EZHIP in lines endogenously expressing this oncogenes results in a drastic loss in tumorigenic capacity that can be rescued upon EZHIP re-expression. EZHIP phenocopies H3K27M and results in K27me3 restriction onto CPG-islands resulting in increased KRAS signaling and an epigenomic profile reminiscent of human embryonic stem cells.

Conclusion: The study of epigenetic modifiers in the pathogenesis and progression of bone tumors such as OS has been largely neglected influenced by the view that that these tumors are mostly driven by genetic mechanisms. In addition to the previously reported oncogenic mutations of histone proteins, we describe a potential role for EZHIP in a subset of Osteosarcomas. In osteosarcoma cell lines, EZHIP induction caused aggressive tumor behavior and significantly shortened animal survival. We conclude that altered levels of epigenetic players such as EZHIP may constitute a novel mechanism of OS pathogenesis and progression and strategies targeting such mechanisms should be considered in the future as treatment options for osteosarcoma patients.

(P 380) NEXT GENERATION SEQUEINCING REVEALS TARGETABLE MUTATIONS IN MULTIPLE SARCOMA HISTOLOGIES

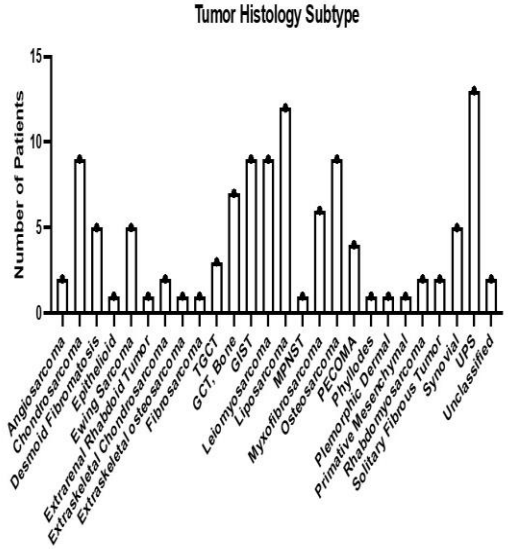
Dixon Dorand, Jr., MD, PhD[1]; Michael J. Robinson, MD[2]; Lauren King, MPH[3]; Emma A. Schremp, MS[2]; Adam Miranda, BS[4]; Ben H. Park, MD, PhD[2]; Leo Luo, MD[5]; Xiao-Ou Shu, MD, PhD[2]; Tuya Pal, MD[2]; Debra L. Friedman, MD, MS[2]; Elizabeth J. Davis, MD[2]; Scott Borinstein, MD, PhD[2], (1) Tennessee Oncology, Nashville, TN, (2) Vanderbilt University Medical Center, Nashville, TN, (3) Vanderbilt University Medical Center, Greenbrier, TN, (4) Vanderbilt University, Nashville, TN, (5) Vanderbilt University Medical Center, Nsahville, TN

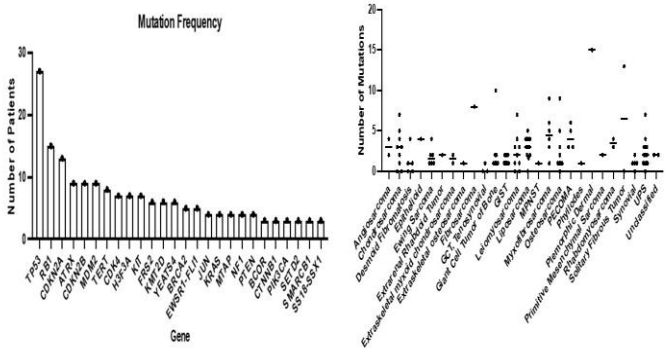
Objective: Sarcomas are a rare and heterogenous group of cancers that arise from bone or soft tissue, and two thirds have poorly defined mutational profiles. Given their rarity, few comprehensive studies have fully characterized mutations and gene expression across sarcoma histologies correlated with clinical outcomes. Further studies are needed to determine the presence of targetable or novel mutations that may improve patient outcomes. In this study, we explored the genomic landscape and clinical actionability of sarcoma mutations from patients enrolled in our CAUSAL (Cohort to Augment the Understanding of Sarcoma survivorship Across the Lifespan) study.

Methods: Between 04/01/2022 and 04/01/2023, 481 participants, treated from 2012 – present with multiple sarcoma histologies were enrolled on CAUSAL. Next Generation Sequencing (NGS) was performed on primary or metastatic tumors from 140 patients to determine DNA mutations within a 648 gene panel. Whole transcriptome RNA sequencing (seq) provided expression profiles and RNA fusions for alternatively spliced products. Further analysis was performed using principal components analysis of RNA seq data to explore correlates amongst sarcoma subtypes. Tumor mutations were queried in ClinVar for relevance to known variants and were assigned to tiers I-IV based on the ESMO scale for clinical actionability of molecular targets (ESCAT). Tier I mutations have drug-mutation matched evidence of actionability while tier IV have only pre-clinical evidence.

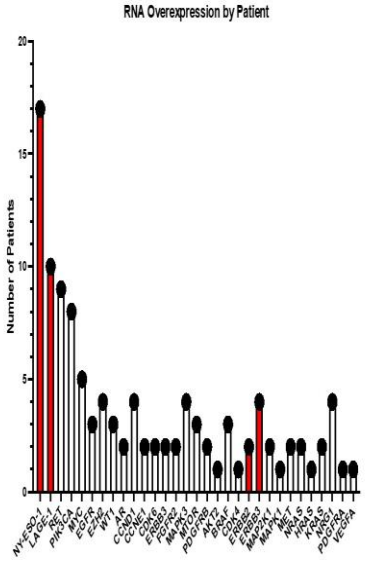
Results: To date, NGS has been completed on 114 tumor samples. Sequenced tumors represented 26 histologies, with the most common ones as follows: undifferentiated pleomorphic sarcoma (15.8%), liposarcoma (9.2%), gastrointestinal stromal tumor (9.2%), osteosarcoma (7.9%), and leiomyosarcoma (7.9%). Of 114 tumors, 101 (88.5%) had at least one mutation detected with a mean frequency of 2.74. TP53 (27/114), RB1 (15/114), and CDKN2A (13/114) were most commonly mutated genes. Mean (std) tumor mutation burden (TMB) was 3.4 m/MB (6.9 m/MB) and one tumor had TMB of 63.7 m/MB. Of the 114 samples, 78 had RNA expression and fusion data available, of which 42 (62%) had anomalous expression changes and 12 had RNA fusions. The most overexpressed genes were NY-ESO-1 (17), LAGE-1 (10), and RET (9); the most under-expressed genes were SMARCB1 (11) and MGMT (6). 31.5% (36 of 114) of patients had potentially actionable DNA mutations, 14 had ESCAT tier I DNA mutations, 4 had tier II, 15 had tier III, and 1 had tier IV. 34.6% (27/78) of patients had potential targets based on RNA expression. 55.2% (63/114) patients had either a potential DNA or RNA target, and 7.9% (9/114) had multiple RNA or DNA targets. 62

Conclusion: NGS revealed potentially actionable targets in half of sarcoma patients based on ESCAT criteria. With ongoing accrual and sequencing of additional tumor specimens future analysis of the CAUSAL cohort will focus on assessing the correlation between targetable mutations and clinical outcomes.


Tumor Histology Subtype
Number of Patients
0
5
10
15
Angiosarcoma
Chondrosarcoma
Desmoid Fibromatosis
Epithelioid
Ewing Sarcoma
Extrarenal Rhabdoid Tumor
Extraskeletal Chondrosarcoma
Extraskeletal osteosarcoma
Fibrosarcoma
TGCT
GCT, Bone
GIST
Leiomyosarcoma
Liposarcoma
MPNST
Myxofibrosarcoma
Osteosarcoma
PECOMA
Phyllodes
Pleomorphic Dermal
Primative Mesenchymal
Rhabdomyosarcoma
Solitary Fibrous Tumor
Synovial
UPS
Unclassified


Mutation Frequency
Number of Patients
0
10
20
30
TP53
RB1
CDKN2A
ATRX
CDH2B
MDM2
TERT
CDK4
HIF-3A
KIT
PBRM1
KMT2D
KMT2A
ARID1A
EIF1AX
ERCC2
JUN
NRAS
MYC
NF1
PTEN
EGFR
CTNNB1
PIK3CA
SETD2
SMARCB1
SS18-SSX1
Gene
Number of Mutations
0
5
10
15
20
Angiosarcoma
Chondrosarcoma
Desmoid Fibromatosis
Epithelioid
Ewing Sarcoma
Extrarenal Rhabdoid Tumor
Extraskeletal chondrosarcoma
Extraskeletal osteosarcoma
Fibrosarcoma
GCT, Bone
Giant Cell Tumor of Bone
GIST
Leiomyosarcoma
Liposarcoma
MPNST
Myxofibrosarcoma
Osteosarcoma
PECOMA
Phyllodes
Pleomorphic Dermal
Primative Mesenchymal
Rhabdomyosarcoma
Solitary Fibrous Tumor
Synovial
UPS
Unclassified

Diagnosis	Gene	Alteration	VAF	Drug	ESCAT Tier	ClinVar Prediction
GIST	KIT	p.W557R	41.6%	Imatinib	I-A	P/LP
GIST	KIT	p.K558_V559del	37.1%	Imatinib	I-A	Uncertain
GIST	KIT	p.A502_Y503dup	22.0%	Imatinib	I-A	New
GIST	KIT	p.V555_K558del	18.1%	Imatinib	I-A	New
GIST	PDGFRA	p.D842V	40.2%	Avapritinib	I-B	P
Liposarcoma	CDK4	CNG		Palbociclib	I-B	N/A
Liposarcoma	CDK4	CNG		Palbociclib	I-B	N/A
PEComa	BRCA2	CNL		Olaparib	II	P
Unclassified	BRCA2	p.S611*	41.6%	Olaparib	II	P
Chondrosarcoma	IDH1	p.R132C	20.1%	Ivosidenib	II-B	P/LP
Unclassified	CDK4	CNG		Palbociclib	III-A	N/A
Liposarcoma	PIK3CA	p.E545K	14.6%	Alpelisib	III-A	P
Chondrosarcoma	IDH2	p.R172S	32.7%	Enasidenib	III-A	P
Osteosarcoma	CDK4	CNG		Palbociclib	III-A	N/A
Fibromatosis	BRCA2	p.T3033fs	9.8%	Olaparib	III-A	P
Fibrosarcoma	NF1	p.S365*	66.9%	Selumetinib	III-A	P
Leiomyosarcoma	BRCA1	p.E1540fs	5.2%	Olaparib	III-A	P
Myxofibrosarcoma	NF1	p.R2258*	67.0%	Selumetinib	III-A	P
Angiosarcoma	PIK3CA	p.E81K	3.4%	Alpelisib	III-B	LP
Angiosarcoma	PIK3CA	p.R38H	9.4%	Alepsib	III-B	LP
Solitary Fibrous Tumor	PTCH1	p.E618*	84.0%	Vismodegib	IV	P



(P 381) THE GENOMIC LANDSCAPE OF CLEAR CELL SARCOMA

Akanksha Farswan, PhD[1]; Fernanda Amary, MD-PhD[2]; Christopher Davies, MSci[3]; Jonas Demeulemeester, PhD[4]; Adrienne Margaret Flanagan, MD-PhD[3]; Daniel Lindsay, MD[5]; Samira Nur, n/a[6]; Nischalan Pillay, MBChB, FRCPath, PhD[3]; Sandra J. Strauss, MD PhD FRCP[7]; Roberto Tirabosco, MD[2], (1) University College London Cancer Institute, London, England, United Kingdom, (2) Department of Histopathology, Royal National Orthopaedic Hospital, Stanmore, UK, London, England, United Kingdom, (3) University College London, Cancer Institute, London, England, United Kingdom, (4) VIB-KU Leuven Center for Cancer Biology, KU Leuven, Vlaams-Brabant, Belgium, (5) Royal National Orthopaedic Hospital NHS Trust, London, England, United Kingdom, (6) UCL cancer Institute, London, England, United Kingdom, (7) UCL Cancer Institute, London, England, United Kingdom

Objective: Background: Clear cell sarcoma of soft tissue (CCS) is a rare soft tissue tumour of tendons and aponeuroses mostly affecting the lower extremities. CCS tumours are quite aggressive with local recurrence rates approaching 40% and metastasis rates of 70%. Since it is a rare cancer, there is limited understanding of the factors leading to disease progression and poor overall survival in CCS. Previous studies have established EWSR1 gene fusions as the genetic hallmarks of CCS. However, the processes which initiate these fusion events are not fully explored in CCS unlike tumours like Ewing sarcoma where chromoplectic loops are known to generate EWS-ERG fusion. All the CCS cases are driven by the same fusion event yet show variation in their survival outcome. The mechanism of cancer evolution in CCS genome is little-known and hence, it becomes imperative to investigate the disease progression in CCS. In this study, we aim to investigate the longitudinal whole genome data of CCS in combination with bulk transcriptomics and methylation data to gain a comprehensive understanding of the CCS genome. Hypothesis: We hypothesize that selection of well-characterized cases along with comprehensive molecular analysis will identify novel correlations between molecular markers and clinical/pathologic features of CCS, potentially leading to identification of therapeutic targets.

Methods: Careful pathological evaluation and clinical annotation was undertaken for 45 CCS samples (28 primaries, 4 local recurrences and 13 metastases) where fresh frozen tumour with matching germline DNA were available. These were sequenced at high resolution and depth using whole genome sequencing (>70X tumour and >30X germline). Similarly, frozen tissue of 37 cases (23 primaries, 2 local recurrences and 12 metastases) underwent bulk RNA sequencing. The resulting fastq files were QC'd ,aligned to the reference hg38 using the fully automated pipeline of bcbio-nextgen toolkit. Bam files from the WGS data were further processed to get the somatic single nucleotide variants, indels, copy number variants and structural variants which underwent consensus mutation calling and manual curation. For RNA-seq data, differential gene expression analysis was done using DESeq2 package on R Studio. For methylation analysis, frozen DNA of 46 CCS samples (30 primaries, 2 local recurrences and 14 metastases) were run on an EPICv2.0 array. The resulting idat files were processed for QC and differential methylation analysis using the sesame package.

Results: Analysis revealed the prevalence of diploidy and low tumour mutation burden across all CCS cases ranging from 0.4 to 3.3 mutations per Mb. There was absence of any specific recurrent non-synonymous mutation, however, the spectrum of somatic non-synonymous mutations identified converged on common signaling pathways like MAPK and PI3K-Akt. Frequent copy number alterations were observed in chromosomes 8, 7, 9 and 17 and unexpectedly identified significant subclonality. Significantly altered recurrent focal copy

number variations were identified at loci- 1p22.1, 1q23.2, 8q24.23, 9p21.3 and 17q25.1. These positions harbor critical genes like MYC, CDKN2A and CDKN2B. >90% samples harbored the EWSR1::ATF fusion followed by EWSR1::CREM and EWSR1::CREB fusions. Unexpectedly, complex structural events of chromothripsis and chromoplexy were identified in 8 CCS patients. Integration of the genomic, transcriptomic and methylation data revealed hitherto unknown patterns of genetic and transcriptional intra and inter tumour heterogeneity.

Conclusion: Despite all CCS showing recurrent driver fusion genes and a paucity of recurrent point mutations, the spectrum of genetic heterogeneity underpinned by subclonal copy number changes, complex structural events such as chromothripsis and chromoplexy is unexpected. Differential gene expression analysis between primary and metastatic tumours showed insights into potential mechanisms associated with metastasis. Evaluation of temporal (primary, recurrence and metastatic) whole genome data will further reveal the evolution patterns in CCS that could provide insights into how to advance treatment strategies, and thereby improving survival in CCS patients. This research was supported by the generous philanthropic gift to UCL by the Edward Showler Foundation. https://www.edwardshowlerfoundation.com/

(P 382) NTRK1/2/3 FUSIONS DETECTION USING RNA-SEQ IN MESENCHYMAL TUMORS REVEALS ULTRA-RARE AND HISTO-SPECIFIC TARGETABLE ALTERATIONS: EXPERIENCE OF AN EXTENSIVE ROUTINE RESEARCH PROGRAM.

Helene Vanacker, MD,MSc[1]; Daniel Pissaloux, PhD[2]; Mehdi Brahmi, MD PhD[2]; Alexandra Meurgey, MD PhD[3]; Marie Karanian, MD PhD[2]; Jean-Yves Blay, MD, PhD[4]; Isabelle Ray-coquard, MD,PhD[4]; Waisse Waissi, MD PhD[2]; François Le Loarer, MD PhD[5]; Sandrine Paindavoine, PhD[3]; Aurélie Houlier, PhD[3]; Nadège Corradini, MD[6]; Franck Tirode, PhD[2]; Armelle Dufresne, MD PhD[6]; Xiaolan Feng, MD PhD[7], (1) Centre Leon Berard, Lyon, Rhone-Alpes, France, (2) CENTRE LEON BERARD AND CANCER RESEARCH CENTER OF LYON (INSERM U1052-CNRS5286), LYON, Rhone-Alpes, France, (3) Centre Léon Bérard, Lyon, Rhone-Alpes, France, (4) Centre Léon-Bérard, Lyon, Rhone-Alpes, France, (5) Institut Bergnonié, Bordeaux, Aquitaine, France, (6) CENTRE LEON BERARD, LYON, Rhone-Alpes, France, (7) Tom Baker Cancer Center, Calgary, AB, Canada

Objective: Since the histo-agnostic approvals of NTRK1/2/3 tyrosine kinase inhibitors (NTRKi), a broad research effort for NTRK1, NTRK2 or NTRK3 fusion transcripts by RNA-seq has been developed. In sarcomas, 5 typically NTRK-fused histotypes have been identified (infantile fibrosarcomas (IFS), lipofibromatosis-like neural tumors(LLNT), ALK/ROS1 wild type inflammatory mesenchymal tumors(IMT), NTRK-rearranged spindle cell neoplasms (NRSCN), and KIT/PDGFRA wild type gastro-instestinal tumors (wtGIST)). Yet, little has been described regarding the prevalence and relevance of NTRK translocations identified in mesenchymal tumors. The objective of this study is to provide an extensive landscape of FFPE samples whole-RNA-seq (WERS) in mesenchymal tumors and to propose a several steps process to ensure relevance of identified NTRK fusions.

Methods: This study was performed as a retrospective translational research program on mesenchymal tumor of pediatric and adult patients from French cancer centers diagnosed between 2016 and March 2023. All diagnosis were histologically and molecularly reviewed by expert pathologists. WERS was performed with FFPE material using TruSeq RNA Access Library Prep Kit (Illumina®, San Diego, USA). Bioinformatics analyses (described in PMID:

35080790) included 4 to 6 different algorithms for fusion detection. A multidisciplinary molecular board reviewed the WERS results in term of technical, biological and clinical interpretations.

Results: WERS was performed in a cohort of 3015 cases composed of suspicions of both malignant and benign mesenchymal tumors which most important subtypes are summarized (Table1). At least 1 fusion of NTRK1, NTRK2 or NTRK3 was reported in n=111 (3.68%) cases bioinformatically. Fourty-three cases were untargetable fusions by TKI due to a loss of kinase domain in the fusion or to a frameshift in the NTRK1/2/3 fused, so remaining 2.25% (68/3015) of the cohort were biologically actionable. Another 11 samples had fusions with criteria of technical false positive results due association of to a very low number of split/span reads associated to inconsistant results in fusion calling algorithm and inconsitant partner. Hence, 1.92% (57/3015) of all tumors had biologically and technically reliable NTRK fusions accounting for 51% (57/111) of the baseline possibly NTRK-fused tumors bioinformatically identified. This cohort (see Figure 1) was composed of a large majority of tumors were pathologist had no clear differential diagnosis and could be classified typical NTRK-fused histological subtypes (11 IFS, 14 LLNT, 9 IMT , 19 NRSCN, 1 GIST). Only 3 sarcomas with a prior clear differential diagnosis by pathologist had a possibly targetable NTRK fusions (1 dedifferenciated liposarcoma with MDM2-amplification, 1 intimal sarcoma of the pulmonary arteria with at leat MDM2-overepression, 1 leiomyosarcoma. All had unsual fusions and , no NTRK inibitor has been administered to these patients with the clinical experience available.

Conclusion: WERS is sensitive to detect NTRK fusions in FFPE mesenchymal tumors although the bioinformatically detected fusions must be filtered for biological or technical irrelevance in order to further identify targetable alterations. The prevalence of possibly targetable NTRK1/2/3 fusions were mainly "histo-specific" to the 5 subtypes of “simple genomic sarcomas”. In these cases, the presence of the fusion could be histologically suspected and/or no clear sarcoma subtype was reach prior to the NTRK identification. Besides, the fusion is extremely rare and clinical activity of NTRKi remains unreported in MDM2-amplified or “complex genomic sarcomas”. Our study may help to refine the NTRK fusion screening strategy in mesenchymal tumors and discuss biases of NTRK fusion detection.

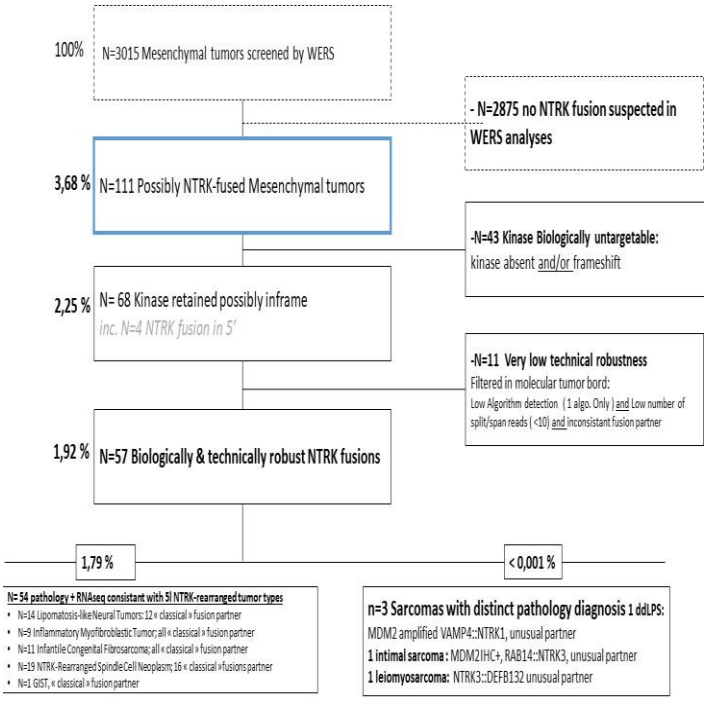


Table 1: Main mesenchymal tumors (n>20 samples)

Subtyping	n=
Unclassified_Tumor_suspiscion_Mesenchymal	410
Unclassified_Sarcoma	353
Undetermined	248
Leiomyosarcoma	136
Unknown_yet	125
Rhabdomyosarcoma_Embryonal	111
Unclassified_Soft_Tissue_Tumor	106
Ewing_Sarcoma	78
Endometrial_Stromal_Sarcoma	70
Dermatofibrosarcoma_Protuberans	62
Inflammatory_Myofibroblastic_Tumor	56
Undifferentiated_Pleomorphic_Sarcoma	54
CIC-Fused_Sarcoma	52
Epithelioid_Sarcoma	49
Tenosynovial_Giant_Cell_Tumor	45
SRF-Rearranged_Perivascular_Myoid_Tumor	40
Histiocytofibroma	39
BCOR-Rearranged	36
Dedifferentiated_Liposarcoma	36
Solitary_Fibrous_Tumor	36
Uterine_Leiomyosarcoma	34
Epithelioid_Hemangioendothelioma	32
Angiosarcoma	30
Myofibroma	30
Gastrointestinal_Stromal_Tumor	29
Rhabdomyosarcoma_Unclassified	29
Hemopigmented_Villonodular_Synovitis	28
Malignant_Peripheral_Nerve_Sheath_Tumor	28
Angiomatoid_Fibrous_Histiocytoma	27
Chordoma	27
Rhabdomyosarcoma_Alveolar	26
Myoepithelial_Tumor	25
Nodular_Fasciitis	25
Fibrous_Lipoblastoma	24
Ovarian_Adult_Granulosa_Cell_Tumor	24
Synovial_Sarcoma	24
Undifferentiated_Sarcoma	24
MAP3K-Rearranged_Myxoma-like_Tumor	23
Ossifying_Fibromyxoid_Tumor	23
Desmoid_Tumor	22
Myxoinflammatory_Fibroblastic_Sarcoma	22
Rhabdoid_Tumor	22
Rhabdomyosarcoma_MYOD1	22
Sarcomatoid_Mesothelioma	22
Histiocyte-Rich_Rhabdomyoblastic_Tumor	21
Myxofibrosarcoma	21

(P 383) AI ANALYSIS SHOWS ENHANCED CCNG1 ONCOGENE EXPRESSION IN SARCOMA TUMORS AND SUPPORTS THE USE OF DELTAREX-G, A CCNG1 INHIBITOR, AS PLATFORM THERAPY FOR ADVANCED SARCOMA

Samantha Jeffrey, BS Pre-Med[1]; Nadezhda Omelchenko, MD[1]; Sant P. Chawla, MD, FRACP[1]; Caroline Eid, BS Pre-Med Student[1]; Zhanna Shekhovtsova, PhD[2]; Kushal Suryamohan, PhD[2]; Elan Younesi, BS Pre-Med[1]; Aleksei Omelchenko, PhD[1]; Amir Ahari, MD[1]; Don Brigham, PhD[1]; Erlinda M. Gordon, MD[3], (1) Sarcoma Oncology Center, Santa Monica, CA, (2) BostonGene Corp., Santa Monica, CA, (3) Sarcoma Oncology Research Center, Santa Monica, CA

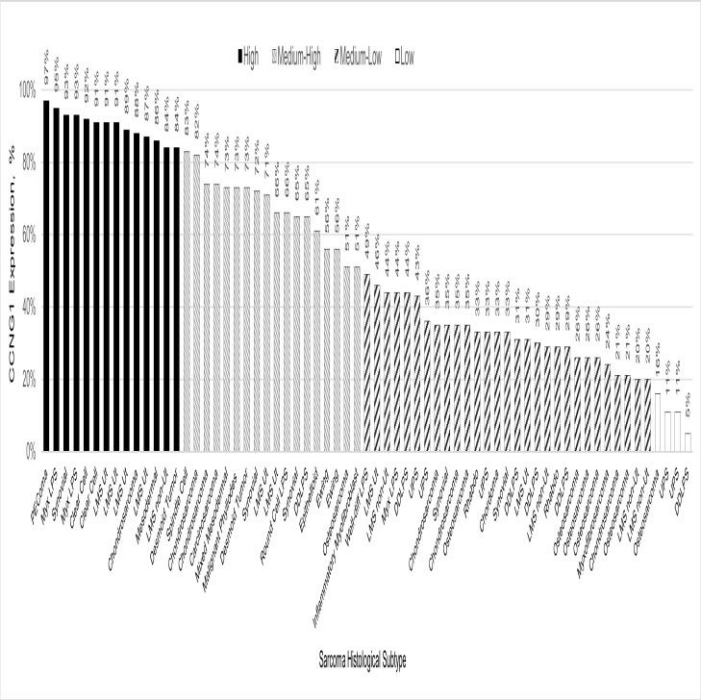
Objective: CCNG1 is an inducible proto-oncogene which is inactive in normal muscle and connective tissues. Enhanced CCNG1 expression has been reported in a variety of solid malignancies including breast cancer, prostate cancer, hepatocellular carcinoma, colon cancer, and recently, in chondrosarcoma.
Expanded access for DeltaRex-G, a CCNG1 inhibitor, is currently open for an intermediate-size population of advanced sarcoma (NCT04091295), based on Phase 1/2 studies wherein DeltaRex-G demonstrated safety and efficacy in advanced sarcoma (n=59), with four (7%) patients reported to be alive > 10 years from DeltaRex-G treatment initiation. However, additional data is needed to identify patients who will respond favorably to DeltaRex-G therapy. Objective: To retrospectively analyze CCNG1 expression in tumors of sarcoma patients who are being followed at our Cancer Center.

Methods: Archived formalin-fixed paraffin-embedded (FFPE) tumor specimens (n=65) from patients with sarcoma who are actively followed at the Sarcoma Oncology Research Center were collected, processed, and subjected to RNA sequencing. Briefly, RNA-seq libraries were sequenced to generate 50 million reads, aligned using Kallisto v0.42.4 to GENCODE v23 transcripts with default parameters. Only protein-coding, IGH/K/L- and TCR-related transcripts were retained for downstream processing, resulting in 20,062 protein-coding genes. Gene expression levels in patients of the reference cohort are categorized as low/medium/high, defined by Low = < 17%; Medium-high = 50%-83%; Medium-low = 17%-49%; High = >83%.

Results: Thirty men and thirty-five women, ranging from ages 16 to 87 years were studied. All tumors showed enhanced CCNG1 expression. Fourteen (22%) tumors showed high CCNG1 expression, 18 (28%) tumors showed medium-high CCNG1 expression, 29 (44%) tumors showed medium-low CCNG1 expression and four (6%) tumors showed low CCNG1 expression (Figure 1). Forty-five (69%) patients had metastatic disease and 20 (31%) patients had localized disease. There was no correlation between CCNG1 expression and disease stage. PEComa had the highest (97%) CCNG1 expression, followed by myxoid liposarcoma, synovial sarcoma, clear cell sarcoma, uterine leiomyosarcoma, chondrosarcoma, mesodermal tumor, non-uterine leiomyosarcoma and desmoid tumor.
Six of 65 (9%) tumors were chondrosarcomas, 3 (50%) of which showed medium-high to high (74-88%) CCNG1 expression and 3 (50%) showed medium-low (21-35%) CCNG1 expression. One patient with metastatic chondrosarcoma was treated with DeltaRex-G under the Expanded Access program. This patient had stable disease and a 25% decrease in palpable chest mass while on DeltaRex-G treatment and lived >3 years after DeltaRex-G treatment initiation. AI analysis of his tumor showed 74% (medium-high) CCNG1 expression.

Conclusion: Taken together, these data suggest that (1) 100% of the analyzed sarcoma tumors have enhanced CCNG1 expression, and (2) DeltaRex-G, a CCNG1 inhibitor, may be

used as the platform therapy upon which targeted therapies/immunotherapies could be added. Phase 2 studies are planned using DeltaRex-G plus an mTOR inhibitor or a tyrosine kinase inhibitor, with or without immunotherapy, for advanced sarcoma.



(P 384) SYSTEMATIC LITERATURE AND CLINICAL TRIAL REVIEW SHOWS TARGETABLE KINASE FUSIONS ARE COMMON IN PEDIATRIC, ADOLESCENT, AND YOUNG ADULT SOFT TISSUE SARCOMAS BUT CLINICAL TRIAL OPPORTUNITIES REMAIN RARE

Lorena Lazo de la Vega, PhD[1]; Sebastian K. Eder, MD, PhD[2]; Ellen Sukharevsky, BS[1]; Zachary A. Kahn[3]; Michaela J. O'Connell, BS[1]; Franziska Wachter, MD[1]; Emily P. Anderson, MD, MSPH[4]; Jenna LaBelle, MS[1]; Kendall Carpenter, MD, MPH[5]; Maria C. Trissal, MD, PhD[1]; Leo Kager, MD[6]; Mariella G. Filbin, MD, PhD[1]; Katherine A. Janeway, MD[7], (1) Dana-Farber Cancer Institute, Boston, MA, (2) St. Anna Children's Hospital, Vienna, Wien, Austria, (3) Dana-Farber Cancer Institute, Hooksett, NH, (4) Massachusetts General Hospital, Boston, MA, (5) Boston Children's Hospital, Boston, MA, (6) St. Anna Children's Hospital and Children's Cancer Research Institute, Vienna, Wien, Austria, (7) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA

Objective: Oncogenic tyrosine kinase fusions (TKFs) have been reported in pediatric, adolescent, and young adult (AYA) soft tissue sarcomas (STS). Clinical trials such as Larotrectinib for NTRK fusions show high response rates to molecularly targeted therapy (MTT) matched to TKFs in pediatric and AYA STSs. TKFs occur across a broad age range of STSs, so they fall into distinct research and care silos. As a result, data on the relationship between TKFs and clinical features (i.e. diagnosis and stage) is distributed across many studies, and

information about response and resistance to MTT is presented in different study types. To address the knowledge gaps resulting from fragmented data, we conducted a systematic review of TKFs reported in pediatric and AYA STSs. We also assessed the availability of clinical trials for pediatric and AYA cancer patients with TKFs.

Methods: A systematic literature search was conducted on PubMed, restricting the publication date to 10 years (2013-2022), during which tumor genomic profiling became more accessible. Search terms included relevant terms for diagnosis, age, and gene names including gene MeSH Terms for BRAF, RAF1, NTRK1-3, ALK, FGFR1-4, MET, RET, and ROS1. Large pediatric sequencing studies including STS patients were also reviewed. Papers were included for abstraction if they reported at least one patient < 30 years old with a TKF-positive STS identified by sequencing or other methods. Data abstracted included report type (case report/series, large sequencing study, molecular pathology methods report, clinical trial study), TKF gene and partner, and diagnosis mapped to ICDOv3.2 codes. Diagnoses occurring in >2 patients were categorized and those in ≤2 were combined in a “Sarcoma, Other” category. When available, additional information for each patient was recorded, including fusion breakpoints, age, stage (local, regional, or metastasis), MTT received, response, and trial enrollment. Clinicaltrials.gov was queried for active or completed interventional trials in the U.S. assessing relevant MTTs in cancer patients.

Results: Our search identified 130 studies (89 case reports/series and 21 pediatric sequencing studies) reporting 401 patients with TKF-positive STSs. TKFs occurred in 22 distinct diagnoses based on ICDOv3.2. Approximately 87% (n=348) of diagnoses were Sarcoma, NOS (8800/3; n=43), spindle cell sarcoma (8801/3; n=55), infantile fibrosarcoma (IFS; 8814/3; n=96), or myofibroblastic tumor, NOS (IMT; 8825/1; n=154). NTRK1-3 were the most common genes involved in TKFs followed, in order of frequency, by ALK, ROS1, BRAF, RET, RAF1, FGFR1-4, and MET. The most common fusion diagnosis pair was ETV6::NTRK3 in IFS (n=66) and TFG::ROS1 in IMT (n=32). Of 60 patients ≥18 years who received MTT, only 17 (28%) were enrolled in a clinical trial. As of Jan 9, 2023, there were 563 trials involving MTTs for TKFs most relevant to the pediatric population. These studies included 47 inhibitors of which only 20 are currently FDA-approved. Patients < 18 years were only eligible for 15% (n=84) of trials (36 completed recruitment and 48 active, recruiting, or not yet recruiting). RET (48%; 10/21) and NTRK (38%; 15/40) inhibitors had the highest percent of trials enrolling patients < 18 years while ERK (6%; 5/79) and MET (2%; 1/41) inhibitors had the least. Notably, there were 173 pediatric and AYA STS patients with an ALK or ROS1 fusion with 73% of patients being < 18 years at diagnosis and only eligible for 24% of ALK or ROS1 inhibitor trials.

Conclusion: To our knowledge, this is the first systematic review of TKFs in pediatric and AYA STSs. While STSs are a heterogeneous group of diseases with distinct histologies, we observe a significant overlap of TKFs across diseases demonstrating a broad need for relevant therapies in these patients. We also observe a discordance between the percentage of pediatric and AYA patients < 18 years old with actionable TKFs and available trials, particularly evident in the ALK and ROS1 TKF-positive patient population.

(P 385) FIRST IN HUMAN PHASE I TRIAL OF A NOVEL DRUG DELIVERY IMPLANT MATRIX WITH SN-38 (CEB-01) FOR RETROPERITONEAL SOFT TISSUE SARCOMA

José Antonio Gonzalez, MD, PhD[1]; Ana Sebio, MD, PhD[2]; Roberto Diaz Díaz-Beveridge, PhD, MD[3]; Juan Francisco Orbis Castellanos, PhD, MD[4]; Rafael López Andújar, PhD, MD[4]; Rosa Álvarez Álvarez, PhD, MD[5]; José Manuel Asencio, PhD, MD[6]; Javier Martin-Broto, MD, PhD[7]; Nadia Hindi, MD, MSc[7]; Montiel Jimenez, PhD, MD[8]; Pedro Villarejo-Campos, PhD, MD[8]; César Serrano, MD, PhD[9]; Nivardo Rodríguez Conde, PhD, MD[10]; Sandra Castro Boix, PhD, MD[10]; Lucas Krauel Gimenez-Salinas, PhD, MD[11]; José A. Tornero García, PhD[12]; Francesc Cano Casas, PhD, MD[13]; Antonio Perez Campos, PhD, MD[14]; Luc Marti, PhD, MD[14]; Joan Beltran, PhD, MD[14]; Antonio López Pousa, MD[2], (1) Department of Surgery, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, Barcelona, Catalonia, Spain, (2) Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain., Barcelona, Catalonia, Spain, (3) Medical Oncology Department, Hospital Universitario y Politécnico La Fe, Valencia, Spain., Valencia, Comunidad Valenciana, Spain, (4) Department of Surgery, Hospital Universitario y Politécnico La Fe, Valencia, Spain., Valencia, Comunidad Valenciana, Spain, (5) Medical Oncology Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain., Madrid, Madrid, Spain, (6) Department of Surgery, Hospital General Universitario Gregorio Marañón, Madrid, Spain., Madrid, Madrid, Spain, (7) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain, (8) Department of Surgery, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain., Madrid, Madrid, Spain, (9) Medical Oncology Department, Hospital Universitario Vall d'Hebron, Vall d'Hebron Institute of Oncology, Barcelona, Spain, Barcelona, Catalonia, Spain, (10) Servicio de Cirugía General y Digestiva, Hospitals Universitario Vall d'Hebron, Barcelona, Spain., Barcelona, Catalonia, Spain, (11) Pediatric Surgery Department. Hospital Sant Joan de Déu, SJD Barcelona, Barcelona, Spain, Barcelona, Catalonia, Spain, (12) Universitat Politècnica de Catalunya (UPC), Barcelona, Spain. Cebiotex S.L., Barcelona, Spain, Terrassa, Catalonia, Spain, (13) Universitat Politècnica de Catalunya (UPC), Barcelona, Spain, Barcelona, Catalonia, Spain, (14) Cebiotex S.L., Barcelona, Spain, Barcelona, Catalonia, Spain

Objective: Local recurrence after radical surgery for retroperitoneal soft tissue sarcoma (RPS) occurs in 41–50% of patients in expert centres and constitutes a major cause of death. We assess the role of CEB-01, a biocompatible and biodegradable membrane implant with PLGA nanofibers entangled with SN-38 (7-ethyl-10-hydroxy-camptothecin), the active metabolite of irinotecan, for a steady delivery, when placed in the surgical bed after RPS resection, to increase local control rates and survival in patients with RPS.

Methods: This is a multicentre, open label, first in human trial (EudraCT: 2018-001026-25, NCT04619056) following a 3+3 dose escalation design, and an expansion cohort at the recommended phase II dose (RP2D) in up to 21 participants. Primary objective is RP2D. Safety, efficacy, time to recurrence (based on RECIST 1.1), biomarkers, pharmacokinetics (PK) are secondary objectives. Efficacy is evaluated using RECIST 1.1. CEB-01 is implanted in the retroperitoneum of the patient immediately after surgery and placed on all surfaces that were in contact with and/or were infiltrated by the tumour (if > 5 cm at staging), including any organs, such as the spleen and great vessels; membranes are fixed with titanium clips to allow for better follow-up of disease in imaging assessments during the trial. If complete resection cannot be performed, CEB-01 is placed to cover all unresected areas.

Results: At present, 11 participants have been enrolled: 4 female / 7 male; mean age 63.45 years (range 40 - 77). Specific histologies include dedifferentiated liposarcoma (LPS) (4 cases), well differentiated LPS (5), liposarcoma-myxoid: 1 and leiomyosarcoma (1). Seven patients had previously unresected primary lesions, and 4 were locoregional relapses. The tumors' longest dimension ranged from 51 to 354 mm (median 152 mm). Grade was 1 in 3 cases, grade 2 in 3 cases, grade 3 in 1 case and unknown in 4 cases. Post-surgery margins were R0 in 8 cases, R1 in 2 cases and unknown in 1 case. CEB-01 was implanted in 3 dose-increasing cohorts: 1st) 2 membranes of 180 cm2 each, 25 mcg/cm2, totalling 9 mg; 2nd) 2 membranes of 180 cm2 each, 50 mcg/cm2, totalling 18 mg and 3rd) 4 membranes of 180 cm2 each, 50 mcg/cm2, totalling 36 mg. No dose limiting toxicity (DLT) has been observed in any of the 3 cohorts. The profile of the adverse events (AEs) is similar to those post-surgery of RPS. Death occurred in 3 patients, due to either abdominal infection or bleeding (both unrelated) or retroperitoneal haemorrhage (related). The expansion phase is ongoing. With a median follow-up of 16.3 months (range, 2.1 to 24.9 months), 2 local recurrences in the implant area have been observed after 5.5- and 8.6-months post-surgery, both with the lower dose (9 mg). Two patients have completed 24 months of follow-up. In 5 ongoing patients no recurrences have been observed after 2.1 to 17.8 months of follow-up (median 16.2 months). PK of SN-38 shows a dose linearity, with a half-life ranging from 287 to 372 hours (29 hours when administered as irinotecan 320 mg/m2), Cmax: 0.76- 3.58 ng/ml (68 ng/ml as irinotecan 320 mg/m2) and AUC 0-t: 128- 377 ng/ml*h (474 ng/ml as irinotecan 320 mg/m2). Blood levels of SN-38 were detectable at least up to 28 days after surgery.

Conclusion: CEB-01 is safe, well-tolerated and no dose-limiting toxicity has been observed at either dosage level. Adverse events are similar to those observed post RPS surgery. Local recurrence in the area of the membrane has occurred in 2 out of 10 patients (both treated with the lower dose). No recurrence has been observed after a median follow-up of 16.2 months in the rest of the ongoing patients. CEB-01 provides a local steady and prolonged release of SN-38, with a much lower Cmax than when SN-38 is administered as irinotecan.

(P 386) GENOMIC PROFILING OF GYNAECOLOGICAL SARCOMAS: REPORT FROM THE AUSTRALIA-WIDE PRECISION ONCOLOGY PROGRAM

Yeh Chen Lee, MBBS BMedSci MPhil FRACP[1]; Damien Kee, MBBS FRACP DMedSc[2]; Frank Po-Yen Lin, MBChB FRACP FAIDH PhD[3]; Subotheni Thavaneswaran, BSc MBBS (Honours) FRACP MMed PhD[4]; Christine E. Napier, MSc, PhD[3]; Michael Friedlander, AM MBChB (Hons) MRCP FRACP PhD[1]; Maya Kansara, BSc PhD[3]; Clare Scott, AM MBBS PhD FRACP[5]; Peter Grimison, MD, PhD[6]; Michelle Harrison, MBBS FRACP[7]; Bo Gao, MBBS FRACP PhD[8]; Alison Davis, MBBS (Hons) MSc FRACP[9]; Philip Beale, BSc MBBS FRACP PhD[10]; Diana Adams, BSc(Hons) BMBCh(Oxon) MRCP AMEC FRACP[11]; Anne Hamilton, MBBS FRACP[12]; Jayesh Desai, MBBS FRACP[13]; Chee Khoon Lee, MBBS FRACP PhD[14]; Mandy L. Ballinger, BAppSc BSc MSc PhD[3]; John Simes, AO BSc (Med) MBBS FRACP SM (Harvard) MD[15]; David Morgan Thomas, MBBS FRACP PhD[16], (1) Prince of Wales Hospital, Randwick, New South Wales, Australia, (2) Austin Health, heidelberg, Victoria, Australia, (3) Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia, (4) The Kinghorn Cancer Centre, St Vincent's Hospital, Sydney, New South Wales, Australia, (5) Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia, (6) Chris O'Brien Lifehouse, SYDNEY, New South Wales, Australia, (7) Chris O'Brien Lifehouse, Sydney, New

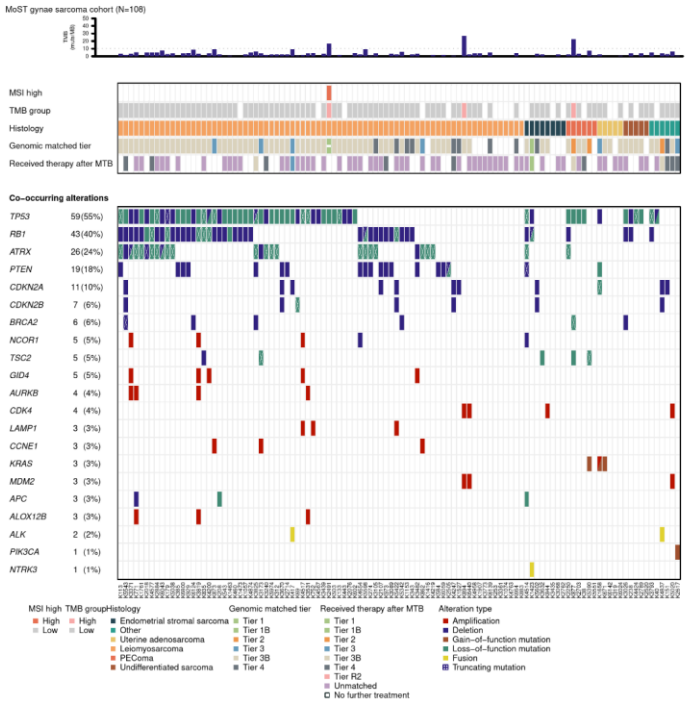
South Wales, Australia, (8) Westmead Hospital, Westmead, New South Wales, Australia, (9) Canberra hospital, Canberra, Australian Capital Territory, Australia, (10) Concord Repatriation General Hospital, Concord, New South Wales, Australia, (11) Macarthur Cancer Therapy Centre, Campbelltown, New South Wales, Australia, (12) Peter MacCallum Cancer Centre, Parkville, Victoria, Australia, (13) Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia, (14) NHMRC Clinical Trials Centre, Kogarah, New South Wales, Australia, (15) NHMRC Clinical Trials Centre, Camperdown, New South Wales, Australia, (16) University of New South Wales, Randwick, New South Wales, Australia

Objective: Metastatic gynaecological sarcomas (GS) have a poor prognosis with limited therapeutic options. We evaluated genomic profiling of GS to identify potentially actionable alterations /biomarkers to guide therapy and to investigate any prognostic implications.

Methods: The Cancer Molecular Screening and Therapeutics Program (MoST, ACTRN12616000908437) is an Australia-wide precision oncology platform for adult patients (pts) with advanced cancers, prioritising rare cancers, to gain access to comprehensive genomic profiling (CGP). Archival tumour specimens were sequenced using a panel-based assay (Illumina TST170 or TSO500, FoundationOne CDX). Variant interpretation was conducted at a weekly molecular tumour board (MTB). Genomic results were tiered using TOPOGRAPH strength of recommendation framework to indicate actionability (https://topograph.info). Pts were followed for subsequent treatment and clinical outcomes. Overall survival (OS) from the date of MTB report issue was estimated by using the Kaplan-Meier method.

Results: Between Sept 2016 and Oct 2022, 108 GS underwent CGP, and were followed for a minimum of 6 months (mo). The majority were ages 45-54 years (n=43, 40%). The overall survival was 17.3 mo (95% CI: 12.8—23.2) The majority received 1 prior line of therapy (range: 0-5) before CGP. Key histologies included uterine leiomyosarcoma (uLMS; n=78, 72%), endometrial stromal sarcoma (n=8, 7%), PEComa (n=6, 6%), and undifferentiated uterine sarcoma and adenosarcoma (n=5 each, 5%). The median number of reported variants was 2.5 (IQR 1-4) and tumour mutational burden (TMB) was 2.6 mut/Mb (maximum 27). Shown in Figure 1, the identified gene alterations include loss-of-function alterations in the TP53 (n=59, 55%), RB1 (n=43, 40%), ATRX (n=26, 24%), PTEN (n=19, 18%), CDKN2A (n=11,10%), BRCA2 (n=6, 6%), and TSC2 (n=5, 5%) genes. In pts with uLMS, TP53 mutation was associated with poorer prognosis (mutated vs wild type, 14.0 vs 28.6 mo; HR 1.92, p=0.045). Overall, 69 (64%) pts received further therapy after CGP, including 16 biomarker-linked therapies and 53 standard of care therapies. Further stratification by actionability tier identified only 11 patients (10%) had biomarker-linked therapies belonging to a clinically active tier group (TOPOGRAPH tier 1-3A). Notable alterations include 2 ALK fusion (1 uLMS harbouring TIMP3-ALK fusion, and 1 inflammatory myofibroblastic tumour harbouring TNS1-ALK fusion), 1 microsatellite unstable (uLMS), 1 EML4-NTRK3 fusion, and 2 TSC2 mutations (2 PEComa); high TMB was observed in 1 each of PEComa and uLMS, but were microsatellite stable. Only 2 pts received biomarker-linked therapies after CGP. Pt with PEComa harbouring a BRCA2 mutation and high TMB (20.3 mut/Mb) was treated on a matched MoST trial of olaparib and durvalumab (ACTRN12617001000392), achieving a complete radiological response and remained progression free at 22.6 mo. Pt with endometrial stromal sarcoma harbouring EML4-NTRK3 fusion received larotrectinib and had stable disease at 35 mo.

Conclusion: Molecular characterization of GS through CGP may be prognostic and can also identify actionable alterations in small subsets of patients that can help delineate this heterogeneous group.



(P 387) CLINICAL CHARACTERIZATION OF PATIENTS WITH LEIOMYOSARCOMA HARBORING THE R337H TP53 GERMINATIVE ALTERATION (LI-FRAUMENI SYNDROME) AND THE PROGNOSTIC IMPLICATION USING A PROPENSITY-SCORE MATCHED COHORT

Fernando Campos, MD[1]; Joyce Lisboa, M.D.[2]; Nathália Carvalho, PhD[2]; Giovana Torrezan, M.D., PhD[2]; Maria Nirvana Formiga, MD, PhD[2]; Celso A L Mello, Ph.D[3], (1) Medical Oncology Department, A.C.Camargo Cancer Center, São Paulo, SP, Brazil, Sao Paulo, Sao Paulo, Brazil, (2) A.C.Camargo Cancer Center, Sao Paulo, Sao Paulo, Brazil, (3) AC Camargo Cancer Center, São Paulo, Sao Paulo, Brazil

Objective: We seek to characterize the clinical presentation of patients (pts) with leiomyosarcoma (LMS) harboring the R337H TP53 germinative alteration (TP53g) and to analyze the survival outcomes compared to a cohort of pts with wildtype R337H TP53 (TP53wt) LMS.

Methods: We retrospectively reviewed consecutive cases of pts diagnosed with LMS in our hospital from January 2000 to January 2023 who were positive for the R337H TP53 pathogenic variant (PV). In addition, constitutional DNA of pts with LMS stored in the biobank were prospectively evaluated for the mutation. The TP53wt cohort was formed by the cases registered in the institutional database added to the negative cases founded by the biobank samples testing. Overall survival (OS) was defined as the time from treatment initiation until death from any cause. Disease-free survival (DFS) was evaluated for pts with localized disease and defined as the time from first treatment until the date of objective disease progression or death from any cause in the absence of progression. Qualitative variables were compared with chi-square and Fisher exact tests. Propensity score was used to match the patients according

to the variables of interest (histology, primary site, age, sex, ECOG, size, extension, grade, neo/adjuvant chemotherapy, neo/adjuvant radiotherapy). Survival was calculated using the Kaplan-Meier method and the curves were compared by the log-rank test.

Results: Ninety-two patients diagnosed with LMS and tested for R337H TP53 status were included of which 30 (32.6%) harbored the R337H TP53 variant. In the TP53g cohort, the median age at diagnosis was 54.5 yo (26–74) and the site of primary lesion was retroperitoneum/pelvis in 23 pts (76.6%). Ninety-three percent of the tumors (n=28) were grade 2 or 3. Most patients (90%) were diagnosed with localized disease. The size of the lesion was 5–10 cm in 9 pts (30%), and 10–15 cm in 12 pts (40%). All the clinical characteristics of included pts and patterns of treatment for localized disease are presented in table 1. At the time of analysis, the median follow-up was 85 months for the whole series (IQR, 51–110 months). Overall, for the 92 pts included in the database, the unadjusted median OS (mOS) was 98 months (95% CI 55.1–140.8), with 122 months (95% CI 60.4–183.5) in TP53g cohort, and 98 months (95% CI not applicable) in TP53wt cohort (p=0.63). After propensity score matching of 60 pts with a 1:1 ratio, demographic, baseline tumor characteristics, and treatment patterns for localized disease were well balanced with no significant differences between the 2 cohorts, except for tumor location, which retained an excess of pts with retroperitoneal tumors in the TP53g cohort, and a higher number of extremities tumors in the TP53wt cohort. In the 1:1 matched population, there was a numerically non-statistically significant difference in mOS between the 2 cohorts: 121 months (95% CI 62.4–179.2) in the TP53g cohort versus 74 months (95% CI 55.5–92.4) in the TP53wt cohort (p=0.31). The median DFS for pts with localized disease at diagnosis was 33 months (95% CI 14.3–51.6) in the TP53g cohort, and 120 months (95% CI not applicable) in the TP53wt cohort (p=0.25).

Conclusion: Our data show that pts with LMS haboring the R337H TP53 germinative PV are more likely to present grade 2/3 tumors located in the retroperitoneum. The presence of the mutation might represent better prognosis for patients in comparison to non-mutated pts, but further study is ongoing to increase the number of pts in the analysis, as well as to compare the prognostic implications of others TP53 PV.

Variable	TP53g cohort n = 30 (%)	TP53wt cohort n = 62 (%)
Age, years (median)	54.4 (26 - 74)	49.5 (19 - 84)
Sex		
Male	16 (53.3)	13 (21)
Female	14 (46.7)	49 (79)
Histology		
Leiomyosarcoma	26 (86.7)	56 (90.3)
Pleomorphic leiomyosarcoma	4 (13.3)	6 (9.7)
Tumor location		
Retroperitoneum/pelvis	23 (76.6)	21 (33.9)
Extremities	4 (13.3)	15 (24.2)
Uterus	1 (3.3)	18 (29)
Other	2 (6.6)	8 (12.9)
Grade		
1	1 (3.3)	7 (11.3)
2	9 (30)	30 (48.4)
3	19 (63.3)	11 (17.7)
Not informed	1 (3.3)	5 (8.1)
Extension		
Localized	27 (90)	49 (79)
Locally advanced/metastatic	3 (10)	13 (21)
Size, cm		
< 5	5 (16.7)	7 (11.3)
5 – 10	9 (30)	30 (48.4)
10– 15	12 (40)	11 (17.7)
> 15	1 (3.3)	9 (14.5)
Not informed	3 (10)	5 (8.1)
Metastatic site		
Lung only	0 (0)	9 (14.5)
Liver only	1 (3.3)	0 (0)
Lung and liver	1 (3.3)	0 (0)
Peritoneum	1 (3.3)	3 (4.8)
Surgery		
Yes	28 (93.3)	60 (96.8)
No	2 (6.7)	2 (3.2)
(Neo)adjuvant chemotherapy		
Yes	10 (33.3)	22 (35.5)
No	18 (30)	29 (46.8)
(Neo)adjuvant radiotherapy		
Yes	9 (30)	19 (30.6)
No	19 (63.3)	33 (53.2)

(P 388) MOLECULAR LANDSCAPE OF ENDOMETRIAL STROMAL SARCOMA IN A LARGE REAL-WORLD PATIENT COHORT

Carlo M. Cicala, MD[1]; Tulio M. Silva, MD[2]; Andrew Elliott, MD PhD[3]; Warren A. Chow, MD[4]; Andreas Seeber, MD PhD[5]; Gina Z. D'Amato, MD[6]; Jonathan Trent, MD, PhD[7]; César Serrano, MD, PhD[8], (1) Vall d'Hebron Institute of Oncology, Barcelona, Catalonia, Spain, (2) Hospital Universitario Vall d'Hebron, Barcelona, Catalonia, Spain, (3) Caris Life Sciences, Phoenix, AZ, (4) University of California, Irvine, Irvine, CA, (5) Medical University of Innsbruck, Innsbruck, Tirol, Austria, (6) Sylvester Comprehensive Cancer Center, Miami, FL, (7) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, (8) Medical Oncology Department, Hospital Universitario Vall d'Hebron, Vall d'Hebron Institute of Oncology, Barcelona, Spain, Barcelona, Catalonia, Spain

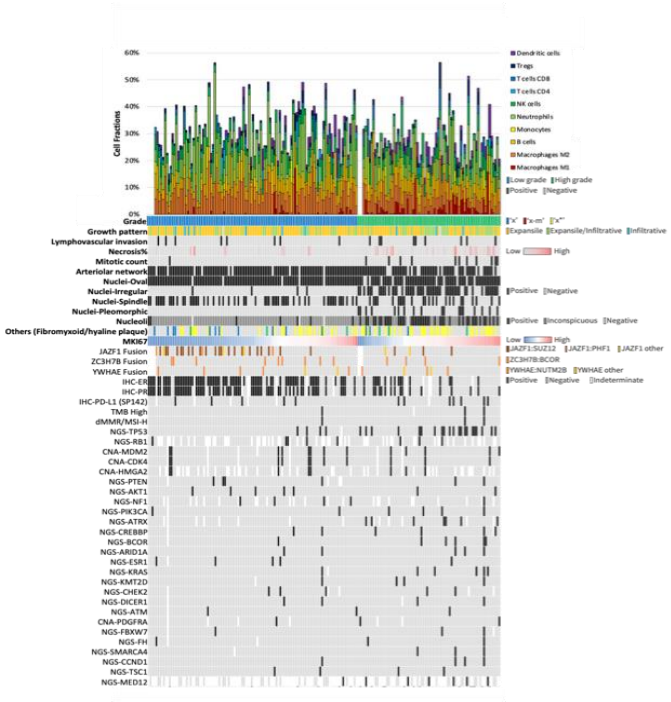
Objective: Endometrial stromal sarcomas (ESS) comprise 10-25% of uterine sarcomas. ESS can be categorized into two types: low-grade (LG-ESS) and high-grade (HG-ESS), each with distinct genomic changes and clinical characteristics. Gene fusions are commonly found in both LG-ESS and HG-ESS, with JAZF1-SUZ12 being prevalent in LG-ESS, while YWHAE-NUTM2 fusion and BCOR alterations, such as ZC3H7B:BCOR fusions or BCOR internal tandem duplications (ITD), are common hallmarks in HG-ESS. However, there is limited knowledge about additional molecular features. Our aim was to perform a comprehensive molecular profiling of the largest ESS cohort so far to uncover novel biological events in these tumors.

Methods: ESS patient samples underwent next-generation sequencing (NGS) of DNA (592-gene, N = 20; whole exome, N = 165) and RNA (whole transcriptome, N = 178) at Caris Life Sciences. All samples were centrally reviewed by an expert sarcoma pathologist. Expression of estrogen (ER) and progesterone (PR) receptors was evaluated by immunohistochemistry (IHC). Proliferation was assessed by KI67 transcriptional expression. Tumor mutational burden (TMB) was calculated by the number of non-synonymous somatic mutations (single nucleotide variants and small insertion/deletions) per mega-base (Mb); a high TMB was defined by a value ≥ 10 mutations/Mb. Tumor microenvironment (TME) was analyzed by RNA deconvolution (quanTIseq). Statistical significance was tested by $\chi 2$, Fisher's exact, or Mann-Whitney U when appropriate.

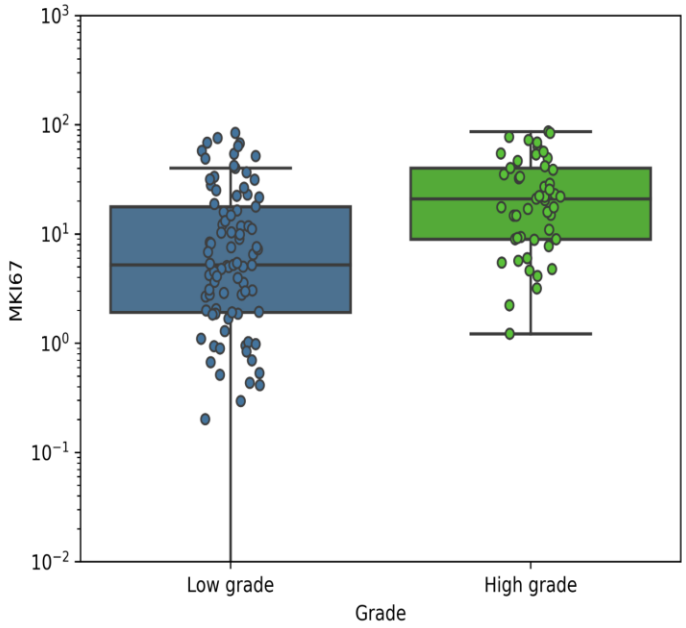
Results: 185 ESS samples (110 LG-ESS, 75 HG-ESS) were analyzed with NGS. Median age was 58 years (range 21-84). Most cases (56% LG and 68% HG) were from metastatic disease. HG-ESS showed higher proliferation (mKi67 RNA expression 21.9 vs 6.7, $p < 0.001$), and more common nuclear pleomorphism and nuclear irregularities. IHC expression of ER and PR was higher among LG-ESS v. HG-ESS samples (73.5% vs 23.2% for ER, $p < 0.001$; 65.0% vs 24.6% for PR, $p < 0.001$).
JAZF1:SUZ12 was the most common translocation in LG-ESS (21%), which was also found in 4% of HG-ESS (4/5 were ER/PR positive). JAZF1:PHF1 fusion was found in 3 LG-ESS, while other JAZF1 fusions occurred in 3 LG-ESS and 2 HG-ESS. ZC3H7B:BCOR fusions were detected in both LG-ESS (N=9, 8%) and in HG-ESS (N=4,6%). Finally, YWHAE:NUTM2B fusion was found in 5 LG-ESS (5%) and 3 HG-ESS (4%); other YWHAE rearrangements were found in 2 LG-ESS and 2 HG-ESS.
Mutational events were uncommon irrespective of ESS grade. Compared with LG-ESS, HG-ESS showed more mutations in TP53 (28.0% v. 3.7%, $p < 0.001$) and MED12 (20.7% v. 2.9%, $p = 0.04$). Mutations in ATRX were found exclusively in HG-ESS (10.1% vs 0.0%, $p = 0.001$). BCOR mutations were detected in 0.9% LG-ESS case v. 6.7% HG-ESS.
Copy-number alterations detected in > 5% of the cases were uncommon and mainly affected MDM2 (7.3%), CDK4 (6.2%), LRIG3 (5.9%) and HMGA2 (5.7%). TMB was globally low

(median 1.0 and 3.0 mut/Mb for LG-ESS and HG-ESS, respectively). dMMR/MSI-H was observed only in 1.6% cases.
TME analysis revealed low T-cell infiltration in both LG-ESS and HG-ESS (< 1% for all T-cell subsets); LG-ESS were enriched in neutrophils (2.0-fold, p = 0.01) and M2 macrophages (1.5-fold, p < 0.001) while the most differentially represented cell fractions in HG-ESS were dendritic cells (5.2-fold, p < 0.001).

Conclusion: This series represents the largest cohort of multi-omic characterization of ESS. Our data confirmed the characteristic presence of JAZF1:SUZ12 fusions in LG-ESS. However, this fusion was also found in HG-ESS cases with retention of ER and PR expression. In addition, both HG-ESS associated with ZC3H7B:BCOR or YWHAE:NUTM2 fusions were also detected in LG-ESS samples. These findings may suggest that some HG-ESS cases might evolve from low-grade tumors. The ER/PR retention in ~ 25% of HG-ESS could provide valuable treatment options.
Compared to LG-ESS, HG-ESS presented more frequently mutations in known tumor-suppressor genes such as TP53, MED12 and ATRX. However, the TMB was low across the whole cohort.
Our works also provides the first description of TME in ESS, indicating globally a low T-cell infiltration for both LG-ESS and HG-ESS, while the relative abundance of neutrophil and M2 macrophage in LG-ESS is of uncertain significance and warrants further investigation.



	Biomarker	P-value (MW)	Q-value (BH)
High grade-Low grade	MKI67	0.000006	0.000006



(P 389) OSTEOBLASTIC-GENE SIGNATURE TO PREDICT SURVIVAL IN PEDIATRIC OSTEOSARCOMA (OS) AND TO FOLLOW PATIENTS TOWARDS LIQUID BIOPSY: A COLLABORATIVE WORK IN OS2006 PROTOCOL.

Natacha ENTZ-WERLE, MD, PhD[1]; Erwan Pencreach, PharmD, PhD[2]; Eric Guerin, PharmD, PhD[3]; Noelle Weingertner, MD[2]; Françoise Françoise, PhD[4]; Anne Gomez-Mascard, MD, PhD[5]; Nathalie Gaspar, PhD MD[6]; Sophie Piperno-Neumann, MD[7]; Perrine Marec-Berard, MD[8]; Marta Jimenez, MD[9]; Monique Dontenwill, PhD[10], (1) University hospital of Strasbourg and UMR CNRS 7021, Strasbourg, Alsace, France, (2) University Hospital of Strasbourg, Strasbourg, Alsace, France, (3) University Hospital of Strasbourg and UMR CNRS 7021, Strasbourg, Alsace, France, (4) UMR_S 1307 Center for Research in Cancer and Integrated Immunology, Nantes, Pays de la Loire, France, (5) Institut Universitaire du Cancer de Toulouse, Toulouse, Midi-Pyrenees, France, (6) Gustave Roussy cancer campus, Paris, Ile-de-France, France, (7) Institut Curie, Paris, Ile-de-France, France, (8) Centre Léon Berard, Lyon, Rhone-Alpes, France, (9) Unicancer, Paris, Ile-de-France, France, (10) UMR CNRS 7021, Strasbourg, Alsace, France

Objective: The survival of pediatric osteosarcomas (OS) is stable worldwide for two decades. The management of OS is lacking new approaches to classify closely the patients and adapt thereafter the chemo-treatments. The objectives of our molecular study, using allelotyping, quantitative PCR (qPCR) and droplet digital PCR (ddPCR), were to determine the survival impact of specific genes previously involved in the bone de-differentiation osteosarcomagenesis (e.g., APC, TWIST1 and CMET). This panel was already identified in the large tumor cohort of the French national protocol OS94, where this 3-gene deregulation was significantly associated to a worst prognosis. By the past, looking in mouse model and combining those gene defects, we were able to reproduce osteosarcomas. The objectives are

now to go further and understand the 3-gene signature significance throughout the recent French national OS2006 protocol.

Methods: Using tumors (e.g., initial tumor biopsies, the paired-tumor resection after neoadjuvant chemotherapy and on circulating DNA at different treatment steps), we applied retrospectively in this OS2006 protocol cohort (144 patients) the same molecular assessments comprising the targeting of those 3 genes with 3 techniques evaluating rearrangements. Targeting the 3 genes, we performed in parallel allelotyping, copy number variations assessment by real-time qPCR and ddPCR. A preliminary cohort of 28 patients with liquid biopsies at diagnosis, before tumor surgery and at the end of chemotherapy was screened by ddPCR with this 3-gene panel.

Results: In terms of results, we were able to determine frequent rearrangements in the regions containing these 3 genes and a statistically significant correlation was made between the presence of a gene rearrangement and a worst outcome. 71% of the entire OS studied population rearranged on at least one of these 3 genes, 34.4% presenting 2-gene rearrangements and 14.6% presented abnormalities in the 3 genes. All rearrangement in the 3-gene panel was correlated with a worst EFS and OS. Using this 3-gene signature in QPCR analyses at a prognostic point of view, the sensitivity was of 96% and specificity of 85%. The predictivity of worst outcome when presenting a rearrangement in 2 or 3 genes is of 92%. No correlation with chemoresponse for any biomarker was observed. Furthermore, on the tumor resection, the persistence or appearance of new clones was significantly correlated to a higher risk of relapse. This 3-gene signature was also usable for residual disease assessment on plasmatic DNA, and we were able to follow closely osteosarcoma patients and their evolution.

Conclusion: In conclusion, this molecular signature seems to be clearly associated with patient outcome and was able to provide evidence that this approach is a powerful tool to be used in pediatric OS follow up.

(P 390) NOVEL ISOFORM DISCOVERY IN SARCOMA USING FULL-LENGTH PACBIO ISO-SEQ

Hyojin Song, PhD[1]; Theodore B. Verhey, PhD[1]; Roger Volden, PhD[2]; Zev Kronenberg, PhD[2]; Michael Monument, MD MSc FRCS(C)[1]; Sorana Morrissy, PhD[1], (1) University of Calgary, Calgary, AB, Canada, (2) Pacific Biosciences, Menlo Park, CA

Objective: Sarcomas can be driven either by genomic translocation resulting in fusion genes, or genomic instability. Both classes of sarcoma have poor outcomes, highlighting the need to better understand these diseases and develop novel therapeutic strategies. One aspect of tumour biology with a high potential to yield new insights into tumour-specific targets is transcriptional diversity. However, commonly used short-read sequencing approaches are insufficiently powered for high-confidence transcript isoform inference. Here, we employ the high-fidelity (HiFi), long-read sequencing PacBio Iso-Seq platform to directly sequence full-length (FL) transcripts and discover novel isoforms. We apply this approach to a sarcoma cohort including (1) alveolar soft part sarcoma (ASPS) and synovial sarcoma (SYNS), both fusion-driven tumour types, and (2) angiosarcoma (ANGS) and undifferentiated pleomorphic sarcoma (UPS), both genetically complex.

Methods: Twelve patient samples were profiled with the PacBio Iso-Seq platform from total

RNA, including 2 SYNS, 3 ASPS (primary & metastasis), 4 ANGS (biopsy, primary, recurrence), and 3 UPS (untreated, primary/neoadjuvant treated) cases. HiFi reads were aligned to the reference genome and cleaned FL reads were clustered and classified into structural isoform categories based on the PacBio IsoSeq3 and Pigeon pipelines. We explored the distribution of isoform categories across the cohort and within individual tumour types, specifically focusing on novel isoforms. Functional enrichment analysis of genes with novel isoforms was carried out using g:Profiler.

Results: We identified a total of 24,130 unique genes with FL read support among all libraries, with an average of 13,400 unique genes/library. Isoforms were classified into 9 different categories (e.g., full- or incomplete-splice match, novel isoforms involving known and/or new splice sites, etc.) and further divided into 14 sub-categories (e.g., alternative 3/5end, multi-exon, intron retention). We focused on genes with novel isoforms that met the following conditions: (i) novel isoforms each had a minimum of 5 FL read counts, and (ii) at least 10% of the FL reads in the gene were supported novel isoforms. This strategy shortlisted 6,774 genes with novel isoforms across the whole cohort. Functional enrichment analysis revealed that novel isoforms are primarily overrepresented in genes associated with cell-substrate and cadherin binding, focal adhesion and junction assembly, cytoskeleton reorganization and cell migration. A further distinction was observed within each tumour subtype, including signalling by Rho GTPases and ECM reorganization in UPS and ANGS, and mRNA splicing terms in SYNS. Additionally, novel isoforms were found for genes in currently ongoing clinical trials in sarcomas (i.e., MAGEA4, CD276) in SYNS samples, and driver fusions were detected in both ASPS and SYNS samples.

Conclusion: We used PacBio Iso-Seq data to comprehensively profile isoform diversity in both fusion-driven and genetically complex soft-tissue sarcomas. Genes with novel isoforms were highly enriched in functions related to cell motility and cell-cell/cell-matrix interactions, indicating that this aspect of tumour biology harbours a high capacity for fine-tuning via transcriptional diversity.

(P 391) THE EXPRESSION OF PVR AND ITS CLINICOPATHOLOGICAL SIGNIFICANCE IN MPNST.

Naoya Nakahashi, MD; Akira Takasawa, MD. PhD.; Kumi Takasawa, Ph.D; Makoto Emori, MD PhD; Makoto Osanai, M.D. PhD, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan

Objective: Malignant peripheral nerve sheath tumors (MPNST) is defined as a relatively rare malignant tumors (3-5% of all malignant soft tissue tumors) derived from nerve sheath cells. It frequently occurs in thigh, hip, and axilla in one's 20s to 50s. The disease is treated multimodally with a combination of chemotherapy and radiation therapy, but the efficacy of each therapy is unknown. At present, there is a lack of effective molecular-targeted drugs, and the development of new therapeutic targets and strategies is required. Transmembrane protein PVR (CD155) has been identified as a member of the Nectin family of intercellular adhesion molecules in epithelial cells and is involved in cell adhesion and differentiation in normal epithelia. On the other hand, it is reported to be aberrantly up-regulated in cancer cells and involved in cell proliferation and invasion. Resent establishment of cancer immunotherapy and the search for new therapeutic targets have focused on the immune checkpoint TIGIT-PVR as a promising therapeutic target. It has been reported that PVR is not only expressed on antigen-

presenting cells but is also aberrantly up-regulated in cancer cells and suppresses cancer immunity. In this study, we aim to clarify the expression pattern, significance and function of PVR in MPNSTs in order to assess its validity as a therapeutic target and to provide a basis for establishing new treatment strategies to the MPNST with limited therapeutic choices.We perform immunohistochemistry of PVR using MPNST surgical materials and analyze its expression pattern. Furthermore, we examine MPNST cell lines to determine whether and how PVR is involved in cancer malignant transformation.

Methods: < Patients and specimens> Surgical specimens obtained from 29 lesions in 22 cases of MPNT during the period from 2004 to 2020 were resected at Sapporo medical university Hospital. The control group consisted of 34 lesions in 34 neurofibroma (NF) cases. < Immunohistochemistry> Primary antibody：monoclonal rabbit anti-PVR antibody (CST), 1:200 Secondary antibody and detection: Dako REAL™ EnVision™ Detection System. Intensity: negative (0), weak (1), moderate (2), or strong (3) Area: 0 (no staining), 1 (1-10%), 2 (11-20%) ～ 8 (71-80%), 9 (81-90%), 10 (91-100%) Immunoreactive score (IRS): i.e., intensity 3 x proportion 10 = immunoreactive score 30, scale of 0-30. Human MPNST cell line: SCC24 ＜PVR KO cells＞ PVR knock out cells were established using the CRISPR-Cas9 system. ＜Omics analysis＞ Proteins were extracted from control and PVR KO cells, and peptide samples were prepared for comparative proteomic analysis. ＜Statistics＞ Statestical analysis were performed using 、t-test、Fisher's exact test、Kruskal-Wallis test、Kaplan-Meier survival curve analysis. All statistical analyses were performed with EZR version 1.32(Saitama Medical Center, Jichi Medical University, Saitama, Japan).

Results: Immunohistochemistry of PVR using MPNST surgical material showed that PVR was highly expressed in MPNST compared to NF. The group with high PVR staining had worse prognosis compared to that with low PVR, but there were no significant differences between them. In PVR KO cell lines, proliferation, colony formation, migration, and invasive capacity were significantly decreased. Comparative proteomic analysis showed that expression of an extracellular matrix (ECM) protein and cell-ECM adhesion-related proteins was significantly decreased in PVR KO cells. As identified by comparative proteomic analysis, Western blotting confirmed expression of ECM and adhesion-related proteins was decreased in PVR KO cells . ECM and adhesion-related proteins were also decreased in culture supernatants, indicating decreased secretion of the proteins from PVR KO cells . Adhesion ability was significantly decreased in PVR KO cells .

Conclusion: Increased expression of PVR was observed in MPNST surgical material. Cell biological analysis confirmed that PVR is involved in proliferation, colony formation, migration, and invasive ability of MPNST cells. It was also shown that PVR is involved in cell-ECM adhesion property including production of an ECM protein itself. These results strongly suggest that PVR contributes to cancer malignant transformation independently of its function as an immune checkpoint. Future analyses will focus on the interaction between cancer immune escape mechanisms and the production of ECM.

(P 392) **IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT OF OSTEOSARCOMA**

Ching C. Lau, MD PhD[1]; Aaron Michael Taylor, PhD[2]; Patrick Ng, PhD[2]; Parveen M. Kumar, PhD[2]; Jeffrey Harder, PhD[3]; Joshy M. George, PhD[2]; Paul Robson, PhD[2]; Masanori Hayashi, MD[4]; Michael Isakoff, MD[1]; Silvia Toledo, PhD[5]; Bang Hoang, MD[6], (1) Connecticut Children's Medical Center, Hartford, CT, (2) The Jackson Laboratory for Genomic Medicine, Farmington, CT, (3) The Jackson Laboratory, Bar Harbor, ME, (4) Children's Hospital Colorado, Aurora, CO, (5) GRAACC, Sao Paulo, Sao Paulo, Brazil, (6) Montefiore Medical Center, New York City, NY

Objective: Osteosarcoma (OS) is the most common malignant bone tumor in the pediatric population, accounting for 6% of all pediatric tumors. It is characterized by a high degree of genomic instability, resulting in numerous copy-number alterations and genomic rearrangements but with no disease-defining recurrent activated mutations. Despite multiple clinical trials using various combination chemotherapy, there has been no significant improvement in prognosis for nearly four decades. Given the diverse genomic landscape of OS and the difficulty of identifying druggable therapeutic targets, the use of immunotherapy appears to be a reasonable alternative approach. However, results from recent clinical trials based on immune checkpoint inhibitors (ICIs) were disappointing. This suggests that there may be additional immune suppressive mechanisms in the tumor microenvironment (TME) in OS other than the PD-1/PD-L1 mechanism that could be interfering with the effects of immunotherapy. Therefore we carried out a detailed analysis of the TME of a small number of OS cases using a combination of single cell RNA sequencing and spatial transcriptomics techniques.

Methods: To better understand the immune microenvironment in OS and guide future therapeutic development, we performed single-cell RNA sequencing (scRNA-seq) using the 10x Genomics Chromium platform on six fresh tumor biopsy samples from pediatric OS patients. Raw data was processed using 10x CellRanger to produce transcript read counts for each cell. After further data filtering based on mitochondrial/ribosomal RNA content and doublet removal using DoubletFinder, counts were normalized using Seurat, and cells were integrated across samples with Harmony. Data was combined with a previously-published OS scRNA-seq cohort of six samples (GSE162454). Two additional OS samples were profiled using 10x Genomics Visium spatial transcriptomics for validation of discovered subtypes and to add spatial context.

Results: Initial clustering identified 16 major cell types based on high expression of canonical cell markers, including macrophages, OS cells, T cells, and granulocytes. Copy number changes in OS cells (identified with inferCNV) revealed multiple subclones in each tumor. Several immunosuppressive cell types were identified via subclustering of major cell types, including neutrophil myeloid-derived suppressor cells (MDSCs), regulatory T-cells, exhausted T-cells, and LAMP3+ dendritic cells. Markers for the cell types found in OS were identified for further validation and cohort expansion using imaging techniques, including Visium spatial transcriptomics. We discovered 9 total spot clusters in the Visium data, and performed deconvolution using the scRNA-seq cell identities to examine colocalization of discovered cell types. Many of the discovered clusters were common between patients and showed consistent cell type proportions. However, we did discover patient-specific differences in the frequency of some cell types, with one sample showing a higher proportion of T-cells along with increased presence of colocalized IFN-stimulated macrophages, and the other sample with a greater presence of neutrophils and MDSCs.

Conclusion: Using single-cell transcriptomics, we were able to discover the presence of

multiple distinct immunosuppressive cell subtypes of neutrophils, T-cells, and dendritic cells. Additionally, spatial transcriptomics revealed multiple similar clusters between samples, and common colocalization of the discovered cell types within those clusters. However, differences in T-cell presence and interferon induction may be indicative of patient-specific immunogenicity of OS. Taken together, these preliminary results confirm the hypothesis of significant immunosuppression in the TME of OS caused by different mechanisms. These findings suggest potential novel immunotherapy targets that could be considered in future clinical trials. Functional validation of some of these targets are in progress.

(P 393) INVESTIGATING THE ROLE OF HDACS IN THE PROGRESSION OF METASTATIC UNDIFFERENTIATED PLEOMORPHIC SARCOMA

Asjah L. Wallace, BS[1]; Joy Ban, PhD[2]; Vijitha Puviindran, BS[3]; Puviindran Nadesan, BS[3]; Nicholas Guardino, BS[3]; Benjamin Alman, MD[3]; David Kirsch, MD, PhD[4], Tomasa Barrientos De Renshaw[1], (1) Duke University, Chapel Hill, NC, (2) Duke Univeristy, Durham, NC, (3) Duke University, Durham, NC, (4) Princess Margaret Cancer Centre/University Health Network, Toronto, ON, Canada

Objective: Undifferentiated Pleomorphic Sarcoma (UPS) is the most commonly diagnosed soft tissue sarcoma amongst adults. About ⅓ of patients will eventually develop metastatic UPS, with the lung as the most common metastatic site. UPS consists of a heterogeneous population of cells which can significantly impact the effect of therapeutic interventions. In mesenchymal tumors, it is poorly understood how these different tumor clones functionally impact tumor evolution, specifically how they may contribute to primary tumor growth and metastasis. In previous studies, histone deacetylases have been identified as drivers of metastasis. My objective is to identify and elucidate the epigenetic mechanisms of histone deacetylases (HDACs) on the metastatic potential of a specific subpopulation of cells driving metastatic UPS.

Methods: We model UPS tumors in mice by the activation of oncogenic KrasG12D and knockout of p53. CRISPR barcoding and fluorescent reporters enable us to label individual tumor clones, providing insight on the clonal dynamics of UPS. To initiate tumorigenesis, an adenovirus containing cre recombinase is injected into the hind limb of the mouse. The tumor bearing limb is amputated and metastasis is observed over a period of time. Using these lineage tracing methods, we revealed that the primary tumors are polyclonal in origin, while the metastatic lesions arise from a single clone. This specific subpopulation of cells that can migrate and form distant lesions are identified as metastatic initiating cells or MCs.

Results: Transcriptomic analysis of the MC and non-MC populations revealed over 800 genes that are differentially expressed in the MC population. Gene signatures associated with poor cancer outcomes, such as CINSARC, were also positively enriched in this population. Whole genome sequencing showed no significant differences between these two groups and the genetic variants specific to the MC population represented less than 2% of the total variants. In addition, copy number alterations were found to be highly similar. These results suggest the role of epigenetic factors in the metastatic potential of this specific cell population. In order to investigate these factors, a CRISPR-Cas9 screening was conducted in vivo where over 400 epigenetic modulator genes were targeted. This screening revealed that histone deacetylases (HDACs) played a regulatory role in the ability of the MC cells to metastasize to the lung. Preliminary drug screening data suggests that HDAC inhibitors, such as Domatinostat, have the

potential to specifically target the MCs in order to inhibit metastasis in UPS.

Conclusion: HDACs have been identified as potential therapeutic targets for metastatic UPS. Previous data has shown that a single subpopulation of cells is responsible for the formation of these distant lesions. Ongoing studies aim to identify which HDACs are important for metastasis in addition to elucidating their mechanism of disease progression and further analysis of the effect of pharmacological inhibition.

(P 394) LEIOMYOSARCOMA INTRATUMORAL MACROPHAGE INFILTRATES ARE ASSOCIATED WITH DISTINCT CLINICAL AND GENOMIC PRESENTATIONS

Alicia A. Gingrich, MD[1]; Hannah Beird, PhD[2]; Randy Chu[3]; Ray Traweek, MD[4]; Brandon Cope, MD[4]; Emily Z. Keung, MD, AM, FACS[5]; Christopher Scally, MD[5]; Kelly Hunt, MD[5]; Keila E. Torres, MD, PhD, MBA, FACS[5]; Christina Roland, MD, MS[5]; P Andrew Futreal, PhD[5]; Andrew J. Bishop, MD[6]; B Ashleigh Guadagnolo, MD,MPH[5]; Devarati Mitra, MD PhD[6]; Ahsan S. Farooqi, MD PhD[7]; Dejka M. Araujo, MD[5]; Maria A. Zarzour, MD[6]; Andy Livingston, MD, MS[5]; Anthony P. Conley, MD[6]; Shalin Shreyaskumar Patel, MD[5]; Vinod Ravi, MD, MS, MBA[5]; Neeta Somaiah, MD[5]; Alexander J. Lazar, MD, PhD[5]; Ravin Ratan, MD[5]; Elise Nassif, MD, MS[6], (1) Department of Surgical Oncology, MD Anderson Cancer Center, Houston, TX, (2) Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA, Houston, TX, (3) MD Anderson Cancer Center, Pearland, TX, (4) MD Anderson, Houston, TX, (5) The University of Texas MD Anderson Cancer Center, Houston, TX, (6) MD Anderson Cancer Center, Houston, TX, (7) MD Anderson Cancer Center, Sugar Land, TX

Objective: Leiomyosarcomas (LMS) are aggressive malignancies of smooth-muscle origin encompassing heterogeneous clinical, pathologic, and molecular diseases. Currently, there exist few targeted molecular options for the treatment of LMS and immunotherapy (IO) has been disappointing. In this study, we examine the gene expression of LMS tumors with a focus on immune infiltrate, specifically macrophages. This cell type constitutes the majority of immune infiltrating cells in LMS and new macrophage targeting drugs are being developed. Our objective was to identify actionable targets for therapeutic intervention in specific subgroups of patients with LMS.

Methods: Tumor samples from a series of LMS patients were prospectively collected and banked for DNA and RNA sequencing as part of the MD Anderson Rare Tumor Initiative and the Patient Mosaic™ Project. Associated clinical and treatment data for the patients were collected and maintained. Differential gene expression (DGE) analysis was done using the DESeq2 package for R and the Benjamini-Hochberg procedure used to control for false discovery rate. Immune cell fractions were estimated using the immunedeconv package for R with the quantiseq method. Inferred prevalence of immune cells by type was then interrogated based on multiple tumor conditions, to include vascular tumors, presence of TP53 mutation, disease status at presentation (primary tumor, local recurrence or metastatic disease), and prior receipt of radiation therapy (RT), chemotherapy or IO before time of sample collection. Survival data was analyzed using the survival package for R.

Results: Analysis included 41 tumor samples from 34 patients (Table 1). Median age at diagnosis was 53 years and 79% of patients were female. Twenty-six tumors were soft tissue (STLMS) and 8 were uterine (ULMS). Of these, 11 (32%) were primary tumors at the time of sampling, 9 (26%) were locally recurrent and 14 (41%) were metastatic. Seventeen tumors were of vascular origin and 16 contained a TP53 gene mutation. Prior to sampling, 29 patients had received chemotherapy, 3 patients had received RT to the site sampled, and 4 patients had received IO.

DGE analysis and immune infiltrate based on RNA signature was completed for all samples (Figure 1) and revealed that immune infiltrates varied across samples despite LMS type (STLMS vs ULMS). Six paired samples from 3 patients demonstrate insight into dynamics of immune infiltrate in response to tumor status or treatment, to include synchronous primary tumor and metastasis, tumor pre- and post- RT and tumor pre- and post- TIL therapy.

Across all conditions studied, the presence of M1 macrophages varied between groups, whereas M2 macrophages were more homogeneous (Figure 2). Significant findings include higher M1 macrophage infiltrates in vascular LMS compared to non-vascular LMS (p 0.0029), lower M2 macrophage infiltrate in patient samples post-IO (p=0.01), and lower M2 macrophage infiltrates in tumors harboring TP53 mutations (p=0.021).

Survival analysis was then performed using available clinical data from the patient cohort to stratify risk by M1 macrophage infiltrate. Patients with M1 infiltrate quantified above the median had reduced overall survival (OS) compared to those that did not (5.25 vs. 9.75 years, P=0.3) (Figure 3). This finding was reproduced in patients with the top quartile of M1 infiltrate (2.32 vs. 12.68 years, P=0.2). DGE analysis was then repeated to stratify for M1 infiltrate, and expression of CD47 “don’t eat me” signal was noted to be increased in the tumors from the higher M1 infiltrate group.

Conclusion: M1 macrophage infiltrate is significantly increased in tumors of vascular origin. OS in tumors with high M1 infiltrate is inferior to tumors with low M1 infiltrate, although this did not reach statistical significance. Increased expression of CD47 within high-M1 infiltrate tumors suggests that macrophage infiltration could be used as a biomarker for therapies such as CD47/SIPRa inhibitors. Future directions include validating these findings in a larger cohort.

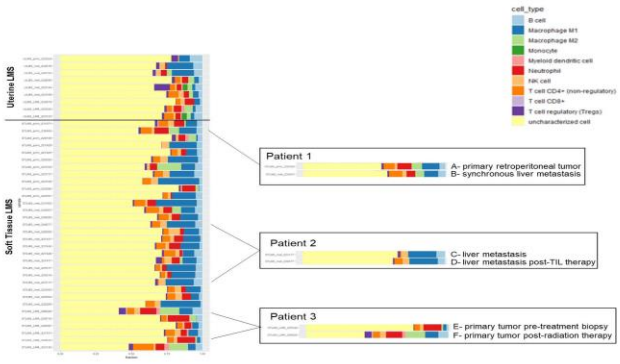
Table 1: Clinicopathologic and Treatment Characteristics of Patients with LMS (n = 34)

Variable	N	Percentage
Age	52.95 (median)	
Sex		
Female	27	79.40
Male	7	20.60
Race		
White	28	82.40
Black	1	2.90
Asian	3	8.80
Other	2	5.90
Ethnicity		
Hispanic or Latino	4	11.80
Not Hispanic or Latino	29	85.30
Status		
Alive with disease	13	38.20
No evidence of disease	6	17.60
Dead of disease	14	41.10
Dead from other cause	1	2.90
Subtype		
Soft tissue LMS	26	
Uterine LMS	8	
Grade		
Grade 1	3	9.10
Grade 2	1	2.90
Grade 3	18	54.50
Unknown	11	33.30
Primary Site		
Head and neck	1	2.90
Trunk	1	2.90
Extremity	2	5.90
Retroperitoneum	18	52.90
Other	10	29.40
Unknown	2	5.90
Size		
	80.5 mm (median)	
Disease State at Collection		
Localized Primary	11	32.40
Locally Recurrent	9	26.50
Metastatic	14	41.20
Tumor of Vascular Origin		
Yes	17	50.00
No	17	50.00
TP53 Somatic or Germline Mutation		
Yes	16	47.05
No	18	52.94
Treatment with Chemotherapy*		
Yes	29	85.29
No	5	14.70
Treatment with Radiation Therapy*†		
Yes	3	8.82
No	31	91.18
Treatment with Immunotherapy*		
Yes	4	11.76
No	30	88.23

*Receipt of chemotherapy, RT and immunotherapy occurred prior to tumor sampling

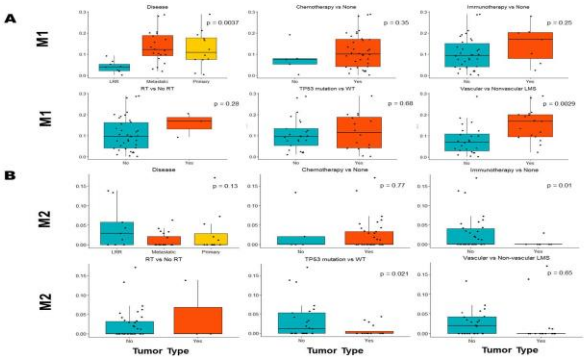
†Receipt of RT occurred directly to the tumor site sampled prior to sampling

Figure 1: Composite Immune Infiltrate of LMS Patient Samples Based on Gene Expression



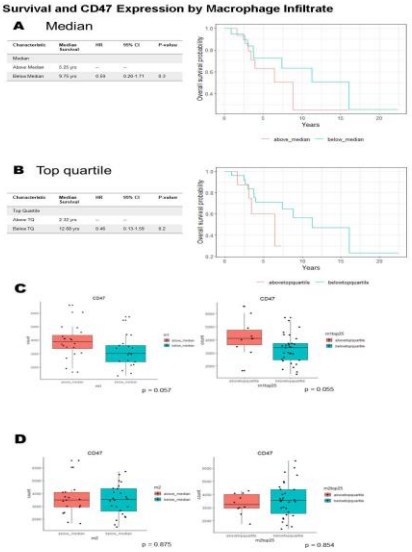
DGE analysis and immune infiltrate based on RNA signature gene expression was completed for all samples. Immune infiltrate was hetergeneous across samples and did not delineate based on tumor type (STLMS vs ULMS). In total, 34 patients contributed to 41 samples resulting in the ability to perform analysis of paired patient samples. Patient 1 had metastatic disease at presentation and was treatment naïve. Sample A is from the primary tumor and sample B is from a synchronous liver metastasis. There is no significant difference between immune infiltrate in the primary tumor versus the distant metastasis. Patient 2 had metastatic disease at the time of sampling following several lines of systemic treatment. Sample C is from a liver metastasis. Sample D is from the same liver metastasis following TIL therapy, with a noted increase in CD4+ T cells (dark orange) within the tumor. Patient 3 had locally recurrent disease at the time of sampling and had been previously treated with doxorubicin and dacarbazine. Sample E is from a pre-surgical biopsy of the recurrent tumor. Sample F is from the surgical specimen following pre-operative RT. There is a marked increase in immune infiltrate for all cell types between samples.

Figure 2: M1 and M2 Macrophage Infiltrate by Tumor Condition



Prevalence of immune cells by type was then interrogated on the basis of multiple tumor conditions. Immune cells studied include B cells, M1 and M2 macrophages, neutrophils, NK cells, CD4 and CD8 T cells, T regulatory cells and myeloid dendritic cells. Conditions compared included vascular vs non-vascular tumors, presence of *TP53* mutation, receipt of RT, systemic or immune targeted therapy, and disease status at presentation (primary tumor, local recurrence or metastatic disease). Of the aforementioned analyses, M1 macrophages were noted to vary across the conditions studied **(Figure 2A)**, whereas M2 was more homogeneous **(Figure 2B)**. Significant findings include higher M1 macrophage infiltrates in vascular LMS compared to non-vascular LMS (p = 0.0029), lower M2 macrophage infiltrate in patients who received immunotherapy (p = 0.01), and lower M2 macrophage infiltrates in tumors harboring TP53 mutations (p=0.021). M1 infiltrate was noted in both the primary and metastatic setting.

Figure 3: Overall Survival and CD47 Expression by Macrophage Infiltrate



Survival analysis was then performed using available clinical data from the patient cohort to stratify risk by M1 macrophage infiltrate. Patients with M1 infiltrate quantified above the median had reduced overall survival (OS) compared to those that did not (5.25 yrs vs 9.75 yrs, HR 0.59, 95% CI 0.02- 1.71, **Figure 3A**). This finding was reproduced in patients with the top quartile of M1 infiltrate (2.32 yrs vs 12.68 yrs, HR 0.68, 95% CI 0.13 – 1.59, **Figure 3B**). DGE analysis was then repeated to stratify for M1 infiltrate and expression of CD47 was noted to be increased in the tumors from the higher M1 infiltrate groups **(Figure 3C)**. Repeat DGE analysis to stratify for M2 infiltrate did not demonstrate a change in expression for CD47 between high-M2 infiltrate and low M2-infiltrate groups (**Figure 3D**).

(P 395) **TARGETING CD44-MEDIATED IRON ENDOCYTOSIS IN DESMOID FIBROMATOSIS**

Sarah Watson, MD, PhD; Stéphanie Solier, PharmD-PhD; Olivier Delattre, PhD; Pierre Gestraud, PhD; Chloé Quignot, PhD; Sylvie Bonvalot, MD, PhD; Dimitri Tzanis, MD, PhD; Sophie El Zein, MD; Laetitia Lesage, n/a; Joshua J Waterfall, PhD; Raphaël Rodriguez, PhD, Institut Curie, Paris, Ile-de-France, France

Objective: CD44 is a transmembrane glycoprotein that is linked to various biological processes reliant on the epigenetic plasticity of cells. During epithelial-mesenchymal transition, CD44 mediates endocytosis of extracellular iron interacting with hyaluronates (HA) and promotes the development of metastases. In desmoid fibromatosis (DF), HA have been shown to colocalize with CD44, and high CD44 is associated with relapse after surgery. Moreover, CD44 is upregulated by the Wnt/□-catenin pathway which is consistently activated in DF. Therefore, we decided to study whether in DF, a CD44-dependent iron endocytosis is occurring and could promote DF cell survival and proliferation.

Methods: Fresh samples of progressive tumors from DF patients collected during surgery were analyzed by flow cytometry, and RNA sequencing (RNAseq) was performed on sorted CD44-high and CD44-low DF cells. Fresh primary DF cells were dissociated and cell viability was measured in vitro after exposure to various iron chelators. A cohort of 40 clinically-annotated pretreatment DF was further studied by RNAseq and multiplex immunohistochemistry.

Results: We observed that all DF cells present high levels of lysosomal iron. The levels of iron and CD44 at the plasma membrane are correlated and are increased in DF tissues compared to healthy adjacent tissues. CD44 and ferritin levels were similarly elevated in both progressive and non-progressive DF by bulk RNAseq or immunohistochemistry in the clinically annotated tumor cohort (n=40). RNAseq on sorted CD44-high DF cells identified an increase of cell cycle-related genes compared to CD44-low DF cells. Finally, the iron chelators ironomycin and deferiprone decreased cell viability of primary DF cells in vitro.

Conclusion: Altogether these data demonstrate that targeting CD44 and iron metabolism could be a valuable strategy in the treatment of desmoid fibromatosis.

(P 396) COMPREHENSIVE WHOLE-GENOME MOLECULAR PROFILING IN BONE AND SOFT TISSUE SARCOMA: MULTI-OMICS ANALYSIS OF 627 CASES ACROSS HISTOLOGIC SUBTYPES

Toshihide Hirai[1]; Kotoe Katayama, n/a[2]; Yuichi Shiraishi, n/a[3]; Akira Kawai, MD, Ph.D[4]; Akihiko Yoshida, MD, Ph.D[5]; Hiroshi Kobayashi, n/a[6]; Toru Hiruma, n/a[7]; Toshiyuki Kunisada, n/a[8]; Yoshihiro Nishida, PhD, MD[9]; Shinichiro Yoshida, n/a[10]; Tsukasa Yonemoto, n/a[11]; Kazutaka Kikuta, MD, PhD[12]; Robert Nakayama, MD, PhD[13]; Masachika Ikegami, n/a[14]; Satoshi Takenaka[15]; Hiroyuki Tsuchiya, MD, PhD[16]; Satoshi Tsukushi, PhD, MD[17]; Kanya Honoki, MD, PhD[18]; Hidetatsu Outani, Dr.[19]; Keisuke Horiuchi, MD, PhD[20]; Hisaki Aiba, n/a[21]; Akio Sakamoto, n/a[22]; Toru Motoi, n/a[14]; Yoshinao Oda[23]; Tadashi Kondo, n/a[3]; Hitoshi Ichikawa, PhD[3]; Tatsuhiro Shibata, n/a[3]; Seiya Imoto, n/a[2]; Koichi Matsuda, MD[24]; Makoto Hirata, MD, PhD[4], (1) National Cancer Center Institute, Tokyo, Tokyo, Japan, (2) The Institute of Medical Science, The University of Tokyo, Tokyo, Tokyo, Japan, (3) National Cancer Center Research Institute, Tokyo, Tokyo, Japan, (4) National Cancer Center Hospital, Tokyo, Tokyo,

Japan, (5) Department of Diagnostic Pathology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (6) Department of Orthopedic Surgery, The University of Tokyo, Tokyo, Tokyo, Japan, (7) Kanagawa Cancer Center, Tokyo, Tokyo, Japan, (8) Okayama University Hospital, Okayama, Okayama, Japan, (9) Nagoya University Hospital, Nagoya, Aichi, Japan, (10) Tohoku University School of Medicine, Tokyo, Miyagi, Japan, (11) Chiba Cancer Center, Chiba, Chiba, Japan, (12) Department of Musculoskeletal Oncology and Orthopaedic Surgery, Tochigi Cancer Center, Utsunomiya, Tochigi, Japan, (13) Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Tokyo, Japan, (14) Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Tokyo, Japan, (15) Osaka International Cancer Institute, Osaka, Osaka, Japan, (16) Kanazawa University, Kanazawa, Ishikawa, Japan, (17) Aichi Cancer Center Hospital, Nagoya, Aichi, Japan, (18) Nara Medical University, Kashiara, Nara, Japan, (19) Osaka University Graduate School of Medicine, Suita, Osaka, Japan, (20) National Defense Medical College, Tokorozawa, Saitama, Japan, (21) Nagoya City University, Nagoya, Aichi, Japan, (22) Kyoto University, Kyoto, Kyoto, Japan, (23) Graduate School of Medical Sciences, Kyushu University, Fukuoka, Fukuoka, Japan, (24) Graduate School of Frontier Sciences, The University of Tokyo, Minato-ku, Tokyo, Japan

Objective: There exist no comprehensive studies based on whole-genome analysis for bone and soft tissue sarcomas, and genomic abnormalities in sarcomas are still largely unknown.

Methods: We included 627 bone and soft tissue sarcomas collected from the Japan Sarcoma Genome Consortium across subtypes which are classified into 2 categories: translocation-related sarcomas (TRS) with specific gene fusions and complex-karyotype sarcomas (CKS) without known fusion. Whole-genome sequencing, RNA sequencing, and methylation array analysis for frozen tumors and paired normal tissues were conducted.

Results: The number of somatic short nucleotide mutations (SNV) and structural variants (SV) was higher in CKS than in TRS. Somatic mutations in cancer-related genes, such as TP53, KMT2C, NF1, IDH1, RB1, and ATRX, were recurrently detected, most of which were more frequent in CKS than in TRS. Non-coding driver mutational analysis revealed frequent SNVs and SVs in the cis-regulatory element of TERT, including a known promoter mutation hotspot (NM_198253.2(TERT):c.-124C＞T). SVs located within 2kb upstream from the transcription start site lead to higher expression of TERT. SV clusters with copy number oscillating pattern which is known as chromothripsis were frequent in all the CKS subtypes (33.3 - 98.2%), while it was rarely seen in most of the TRS subtypes. Chromothripsis underlay 76.6% of focal amplification events in oncogenes and 27.7% of copy number loss in tumor suppressor genes, which were higher than the reported proportions in a study of pan-cancer analysis of whole genomes (PCAWG). We detected a varied range of telomere length (TL) not only among tumor subtypes but among samples of a specific subtype in high- and intermediate-grade sarcomas, whereas a little variation of TL was seen in low-grade and benign tumors. TL was longer in cases with loss-of-function aberrations in ATRX, while gain-of-function alterations in TERT were not associated with TL gain. A clustering analysis based on methylome data for CKS did not make isolated clusters according to the subtypes. An integrated analysis of methylome and expression analysis divided cases of CKS into 4 clusters.

Conclusion: We elucidated the molecular characteristics of bone and soft tissue sarcomas across histologic subtypes by a multi-omics study.

(P 397) A MESENCHYMAL TISSUE LANDSCAPE TO STUDY OSTEOSARCOMA TUMOR HETEROGENEITY

Kevin Murgas[1]; Corey Weistuch, PhD[2]; Danh D. Truong, PhD[3]; Joseph A. Ludwig, IV, Ph.D.[4]; Allen Tannenbaum, PhD[5], (1) Department of Biomedical Informatics, Stony Brook University, Stony Brook, NY, (2) Department of Medical Physics, Memorial Sloan Kettering Cancer Center, New York, NY, (3) The University of Texas MD Anderson Cancer Center, Houston, TX, (4) Department of Sarcoma Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, (5) Departments of Computer Science and Applied Mathematics and Statistics, Stony Brook University, New York, NY

Objective: Osteosarcomas (OS) are a heterogenous class of bone tumors that can occur both in children and adults. OS tumors exhibit complex karyotypes and diverse genetic alterations, although few common pathognomonic drivers have been identified. The diversity of genetic drivers in OS poses a major challenge for treatment strategy because each tumor may possess unique therapeutic vulnerabilities. Clinically, OS tumors are typically classified based on histological assessment including apparent predominant cell types (osteoblastic, chondroblastic) and relative differentiation status (low-grade, high-grade). However, a common feature of OS is that tumor cells can exhibit a range of differentiation states that may include multiple lineages (osteogenic, chondrogenic, adipogenic, etc.) derived from mesenchymal stem cells (MSCs). Instead of mutation-based drivers, differentiation states may depend on epigenetic and transcriptional changes that relate to normal cell differentiation resembling osteogenesis or other mesenchymal differentiation. Importantly, OS might arise from disrupted normal differentiation of MSCs or, alternatively, from a dedifferentiation of mature cells. In order to sufficiently investigate how OS relate to normal mesenchymal differentiation, we first characterized a Mesenchymal Tissue Landscape (MTL) based on single-cell gene expression dynamics across time courses within multiple differentiation lineages (osteogenic, adipogenic, chondrogenic). Then, we utilized gene expression signatures learned from MTL to quantitatively assess differentiation states and lineage heterogeneity with a cohort of OS patient-derived xenografts and OS tumors. Herein, we describe the results of these analyses and highlight certain novel findings relating to tumor heterogeneity in OS.

Methods: First, we collected single-cell RNA-sequencing (scRNA-seq) data from three patient-derived xenograft (PDX) models of OS. We additionally incorporated a publicly available scRNA-seq dataset of 11 human OS tumors. Each PDX and tumor was classified based on a pathologist assessment as osteoblastic or chondroblastic in origin. We applied gene expression analysis to determine cell clusters and marker genes of each PDX, including known marker genes of osteogenesis and chondrogenesis. Pathway analysis was performed to assess molecular pathways expressed in each cell cluster. Next, we developed a tissue-engineered model of mesenchymal cell differentiation using biochemical and biophysical cues (including polyacrylamide hydrogel stiffness) to direct differentiation into two lineages: high stiffness for osteogenic, low stiffness for adipogenic. Cells were cultured and collected at multiple time points until terminal differentiation, and for each time point scRNA-seq was performed to measure gene expression. Additionally, a publicly available dataset containing a similar single-cell time course of chondrogenic differentiation was integrated along with our experimentally generated lineages, resulting in a MTL atlas containing three mesenchymal lineages: osteogenic, adipogenic, chondrogenic. With the MTL gene expression data, clustering and differential expression analysis were used to identify lineage-specific marker genes of early and late differentiation. Then, archetype analysis based on nonnegative matrix factorization was applied to define temporal gene expression signatures corresponding to lineage-specific mesenchymal differentiation states. The differentiation state signatures learned from MTL were

used to directly estimate the composition of differentiation states within each OS PDX and OS tumor as a means of quantitatively assessing intratumor heterogeneity. Further, we applied these signatures to a dataset of bulk RNA-seq data in pediatric OS (TARGET-OS study) and performed survival analysis (multiple Cox regression) with respect to the estimated archetype compositions.

Results: We observed 15 distinct subpopulations of OS PDX cells, some of which were PDX-specific and others which were shared across multiple PDX suggesting some conserved states among different OS PDXs. We observed significant heterogeneity in osteoblastic markers (RUNX2, CDH11, SPP1) expression across the PDXs. All PDXs exhibited a subpopulation of proliferative cells expressing cell cycle markers MKI67 and TOP2A. In one PDX classified as chondroblastic (SA98), we identified expression of several fibroblastic and chondroblastic marker genes indicative of a mixed phenotype. In our experimentally generated mesenchymal differentiation atlas (MTL), we observed a clear trifurcation into three distinct lineages: osteogenic, adipogenic, chondrogenic. Early time points comprised largely undifferentiated and proliferative cells, marked by cell cycle markers and MSC-specific markers. YAP/TAZ regulated genes were enriched in cluster MSC-H versus MSC-L. Cells at later time points were committed to one of the three lineages, with corresponding markers of early and late differentiation specific to each lineage. Archetype analysis determined 12 distinct gene signatures that corresponded to lineage-specific and time-ordered expression dynamics observed in the MTL. We used these signatures to estimate the proportion of each differentiation state within OS PDX and OS tumor gene expression. Importantly, the quantification of osteogenic and chondrogenic fractions of each tumor agreed closely with pathologist labels of predominant cell of origin, suggesting that gene expression analysis with reference to normal mesenchymal differentiation can quantify tumor heterogeneity in agreement with histological assessment. Finally, estimated compositions in bulk OS gene expression were correlated with survival, indicating a more well-differentiated signature was associated with improved outcome.

Conclusion: In our study, we apply a novel paradigm to study OS tumor heterogeneity by constructing a multi-lineage single-cell mesenchymal differentiation landscape. With this framework, multiple differentiation states corresponding to each lineage were able to be resolved corresponding to gene expression dynamics in normal mesenchymal differentiation. Furthermore, novel gene signatures were defined corresponding to a decomposition of temporal- and lineage-specific differentiation states. Importantly, the signatures learned from the mesenchymal landscape were shown to be effective in estimating the cell lineages and differentiation states within OS PDX and tumor data. Our findings indicate that OS tumors exhibit a high degree of intratumor heterogeneity as well as highly variable compositions of cell subpopulations among different OS tumors. Critically, the compositions estimated from gene expression agreed closely with pathologist labels of predominant cell type, validating the accuracy of the signatures and further allowing quantitative estimation of OS heterogeneity. Compared to a single histological label, the proposed method offers greater information by quantifying multiple subpopulations and can reveal mixtures of multiple cell types. Lastly, we demonstrated the differentiation signatures could be applied to estimate compositions of bulk tumor data, wherein a well-differentiated signature was correlated with improved survival outcome. This approach has potential to inform treatment strategy, as dominant cell populations might influence outcomes or require concurrent treatment strategies.

(P 398) INTEGRATED MULTI-OMICS APPROACH REVEALED TUMOR-ASSOCIATED TERTIARY LYMPHOID STRUCTURE LANDSCAPE IN RETROPERITONEAL LIPOSARCOMA

Ping Tao, Postgraduate/Master[1]; Zhenyu Wang, Postgraduate/Master[2]; Jun Chen, MD, PhD[3]; Jiongyuan Wang, Postgraduate/Master[4]; Peidang Fan, Postgraduate/Master[2]; Weiqi Lu, Postgraduate/Master[4]; Lijie Ma, Postgraduate/PhD[5]; Chenghua Luo, MD, Postgraduate/PhD[6]; Yong Zhang, Postgraduate/PhD[4]; Hanxing Tong, Postgraduate/PhD[4], (1) Department of Laboratory Medicine, Shanghai TCM-Integrated Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, Shanghai, Shanghai, China (People's Republic), (2) School of Medicine, Anhui University of Science and Technology, Huainan, China, Shanghai, CA, (3) Retroperitoneal Tumours Centre, Peking University International Hospital, Beijing, China, Beijing, Beijing, China (People's Republic), (4) Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai, China, Shanghai, CA, (5) Department of Liver Surgery, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, Shanghai, CA, (6) Retroperitoneal Tumours Centre, Peking University International Hospital, Beijing, China, Beijing, CA

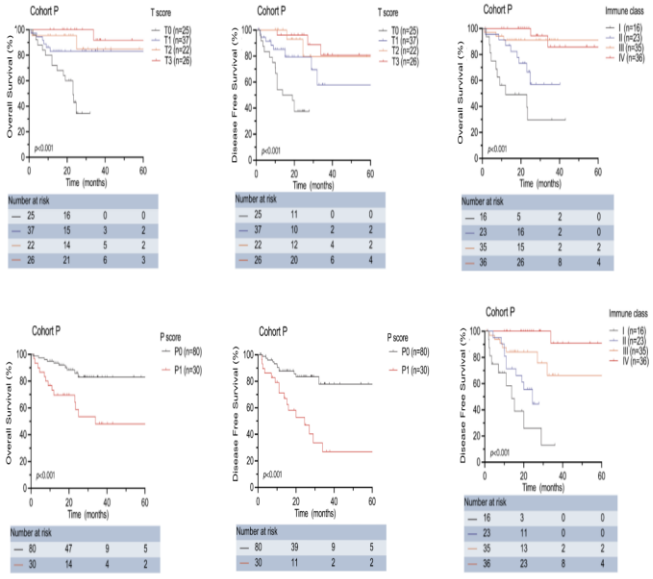
Objective: Retroperitoneal liposarcoma (RPLS) is a rare type of mesenchymal tumor, which is characterized by challenging surgical treatment, immune-desert, dismal immunotherapy response and high local recurrence rate. The presence of tertiary lymphoid structure (TLS), like ectopic lymphoid aggregates discovered in tumor immune microenvironment (TIME), was found associated with favorable patient survival and response to immunotherapy. However, the mechanisms underpinning its clinical activity are unclear. Thus, we aimed to investigate the prognostic value and spatial distribution and functional involvement of TLS in RPLS.

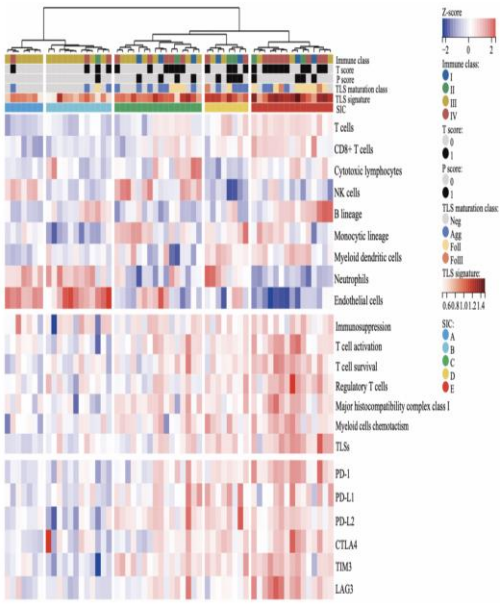
Methods: 330 patients were retrospectively enrolled into five independent cohorts (Cohort-Primary (n=110), Cohort-Relapse (n=76), Cohort-Evolution (n=10, Same patient with well-differentiated liposarcoma (WDLPS) in primary tumor and dedifferentiated liposarcoma (DDLPS) in relapse tumor) Cohort-Validation (n=76) and Cohort-TCGA (n=58)) from the largest two retroperitoneal tumor research center of China and TCGA database. Sc-RNA seq (n=4) and spatial transcriptome seq (n=2) were performed for the estimation of TIME based on treatment-naive samples. Transcriptomic profiles and corresponding clinical information of 309 cases were enrolled from two public databases, including Cohort-TCGA (n=58), Cohort-GSE30929 (n=92), Cohort-GSE21050 (n=59), Cohort-GSE21122 (n=50) and Cohort-FD (n=50). TLS was quantified at three distinct anatomic subregions (intra-tumor, invasion margin and peri-tumor) and correlated with overall survival (OS) and disease free survival (DFS) by Cox regression and Kaplan-Meier analyses. Multiplex immunohistochemistry (mIHC) was performed to characterize and validate the spatial composition of TLS in another treatment-naive RPLS cohort (n=16) and neoadjuvant chemotherapy RPLS cohort (n=12).

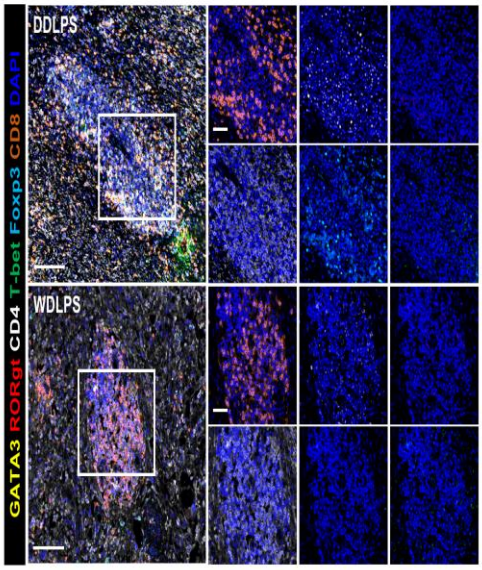
Results: Quantitative and qualitative scoring system of TLS was established for the intra-tumor region (T score), invasion margin region (IM score) and peri-tumor region (P score), respectively. In Cohort-Primary, high T score was positively associated with favorable OS ($p=0.003$) and DFS ($p< 0.001$), whereas high P score predicted dismal OS ($p=0.008$) and DFS ($p=0.001$) (Figure 1). Additionally, the joint scoring system of T and P scores stratified RPLS into four immune classes with distinct TLS distribution patterns and prognoses ($p< 0.001$) (Figure 1). The C-index of the immune class was significantly higher than the TNM staging system (0.798 vs.0.62, $p=0.005$). These results were also validated in Cohort-Relapse and in concordance with the image-omic explanations in Cohort-TCGA. Intriguingly, the spatial

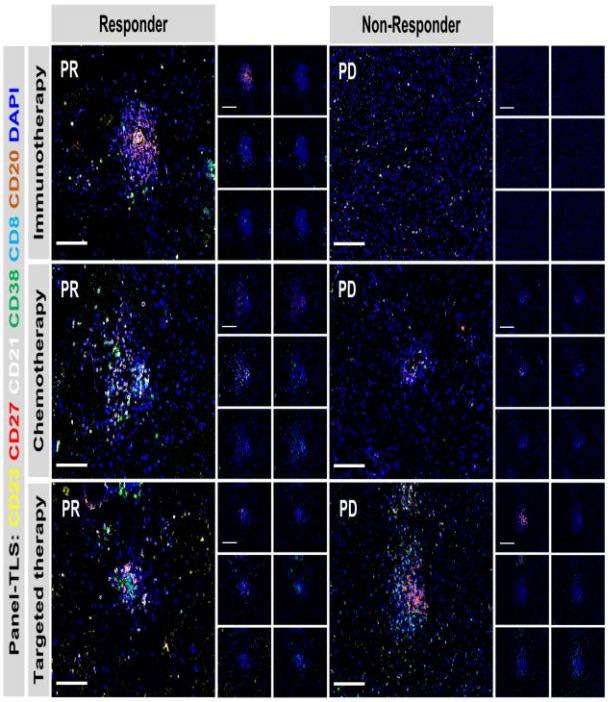
distribution and abundance of TLS indicated a high degree of consistency between H&E evalution and transcriptomic level (Figure 2). Importantly, mIHC discovered that regulatory T cells (Tregs) were significantly increased in intra-tumoral TLS in DDLPS compared with those in WDLPS, displaying an immunosuppressive pattern (Figure 3). Interestingly, neoadjuvant chemotherapy could block this status of immunosuppressive, induced TLS formation and restored the antitumor immune balance in responsive patients, whereas the nonresponsive patients deteriorated into a more suppressive one (Figure 4). Strikingly, significant more CD38+ plasma cell could be found in responsive patients, compared with nonresponsive patients (Figure 4). sc-RNA seq and ST analysis further revealed significant intra-tumoral and inter-tumoral TIME heterogeneity and identified the underlying transcriptomic programs driving each phenotype.

Conclusion: Collectively, our study provides a high-resolution map of the TIME with treatment-naive and neoadjuvant chemotherapy RPLS, including the spatial distribution, composition and immunophenotype. We further uncovers the critical role for TLS-centered antitumor immunity. The results suggested that regulatory T cells might play an essential role in determining functional heterogeneity of TLS. Remarkably, the spatial distribution and abundance of TLS gradually decreased in intra-tumor area, whereas increased in peri-tumor area within the malignant progression from WDLPS to DDLPS. TLS located within distinct regions of TME presented opposite prognostic influences, and the immune classification was able to fuse these heterogeneous modalities to predict varying prognoses, even compared with TNM staging system. Efficient neoadjuvant chemotherapy can induce TLS formation and restore the antitumor immune balance in RPLS.




Z-score
-2 0 2
Immune class
I score
P score
TLS maturation class
TLS signature
SIC
T cells
CD8+ T cells
Cytotoxic lymphocytes
NK cells
B lineage
Monocytic lineage
Myeloid dendritic cells
Neutrophils
Endothelial cells
Immunosuppression
T cell activation
T cell survival
Regulatory T cells
Major histocompatibility complex class I
Myeloid cells chemotaxism
TLSs
PD-1
PD-L1
PD-L2
CTLA4
TIM3
LAG3
Immune class:
I
II
III
IV
T score:
0
1
P score:
0
1
TLS maturation class:
Neg
Agg
Foll
FolII
TLS signature:
0.6 0.8 1.0 1.2 1.4
SIC:
A
B
C
D
E


DDLPS
WDLPS
GATA3 RORgt CD4 T-bet Foxp3 CD8 DAPI



(P 399) CRITICAL MOLECULAR PATHWAYS ASSOCIATED WITH DIFFERENT HISTOPATHOLOGY FEATURES AND DETAILED PATTERNS OF IMMUNE CELL INFILTRATES IN EPITHELIOID HEMANGIOENDOTHELIOMA (EHE)

Tom Wei-Wu W. Chen, MD, PhD; Chia-Lang Hsu, PhD; Tom Wei-Wu W. Chen, MD, PhD; Jen-Chieh Lee, MD, PhD, National Taiwan University Hospital, Taipei, Taipei, Taiwan (Republic of China)

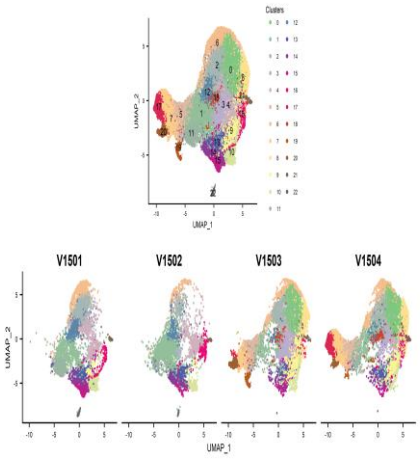
Objective: While the presence of specific gene rearrangements is pathognomonic for EHE, its histopathology is heterogeneous and affects prognosis. Critical molecular pathways associated with prognostic histopathological features of EHE remain elusive. Furthermore, understanding the context of the tumor immune microenvironment in EHE is also limited. We intended to uncover the critical molecular pathways based on histopathology features and the pattern of immune cell infiltrates in EHE.

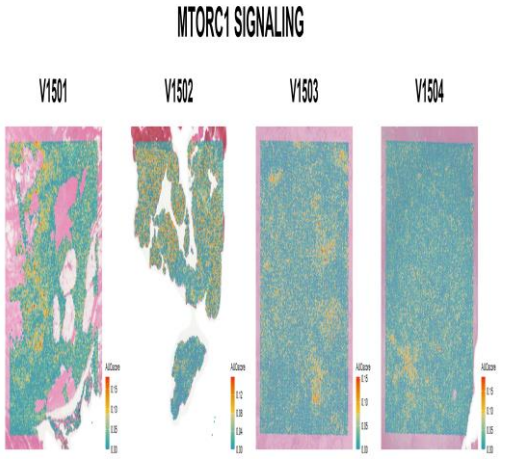
Methods: The samples of EHE with different primary sites and gene rearrangements were selected. The spatial transcriptome of each FFPE sample was analyzed using the Visium slide. Integration was performed on four spatial transcriptome datasets, and graph-based clusters were identified. To calculate the pathway expression intensity of each spot, AUCell was applied to the Hallmark gene-set from MSigDB. Additionally, XCell was utilized to estimate the cell compositions of each spot.

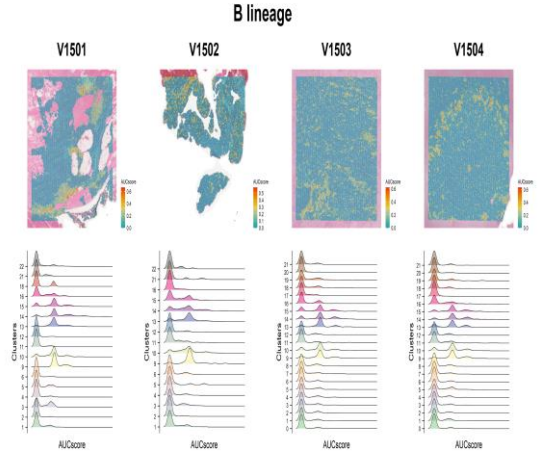
Results: Four EHE patients (F:M 2:2, the median age at diagnosis 41) with two fusion types (CAMTA1 fusion in 3, TFE3 fusion in 1) and three different primary sites (liver in 2, extremity,

pleura) were included. The mean profiled genes was > 18,000, and the spots covered by tissues ranged from 5,887 to 14,334. CD31 and CD34 were homogeneously expressed in the tumor parts, while ERG distribution was patchy. Among 22 identified spot clusters (Fig. 1), three clusters (supercluster A, SA) represented solid sheet pattern EHE tumor cells, and two clusters (supercluster B, SB) were in accordance with the locations of EHE tumor cells with papillary features and/or necrosis. Pathways associated with mTORC1 (Fig. 2), KRAS/MEK, angiogenesis, hypoxia, inflammation, interferon-alpha response, TGF-B were upregulated in SB compared to SA. NOTCH upregulation in SB was only found in CAMTA1-rearranged EHE. Between the sub-clusters of SB, cluster 6 was more commonly associated with necrosis and significantly upregulated pathways of mTORC1, angiogenesis, STAT3/5, epithelial-mesenchymal transformation, and KRAS/MEK pathways. Regarding immune cell infiltrates, B cells were the most prominent immune cell observed. While in CAMTA1-rearranged EHEs, the B cells were located at the invasive border of the tumor, diffuse B cell infiltration was observed in the liver EHE with TFE3 rearrangement (Fig. 3). More study details will be presented at CTOS.

Conclusion: Spatial transcriptomics showed that different histopathology components of EHE have different molecular pathway activation. EHE with different gene arrangements may have differential downstream molecular pathway activations and immune cell infiltration patterns. The differentially activated pathways or immune cells associated with specific prognostic features provides novel insights into selecting future treatments for EHE patients.







(P 400) THE LANDSCAPE OF GENOMIC ALTERATIONS FROM 1494 SOFT TISSUE AND BONE SARCOMAS AT MD ANDERSON CANCER CENTER

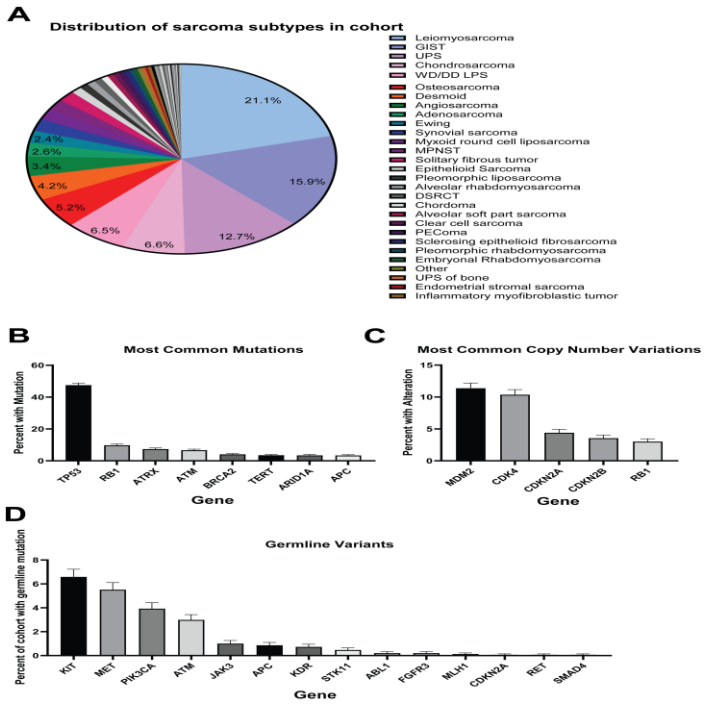
Ryan A. Denu, MD, PhD[1]; Kenna R. Shaw, PhD[1]; Shreyaskumar Patel, MD[2]; Anthony P. Conley, MD[1], (1) MD Anderson Cancer Center, Houston, TX, (2) The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: Sarcomas comprise a relatively rare cancer type derived from connective tissues, though within this cancer type is a heterogeneous group of diseases with nearly 100 different histologic subtypes identified to date. We explored the landscape of genomic alterations and their potential clinical actionability in soft tissue and bone sarcomas with available clinical sequencing at MD Anderson Cancer Center.

Methods: This is a retrospective review of 1494 soft tissue and bone sarcoma cases seen at MD Anderson Cancer Center between 2007 and 2022 with available clinical sequencing data. Most patients had in-house clinical sequencing at MD Anderson. The MD Anderson Precision Oncology Decision Support (PODS) was used to assess for clinical actionability of genomic alterations.

Results: The cohort was mostly comprised of leiomyosarcoma (21.1%), gastrointestinal stromal tumor (15.9%), undifferentiated pleomorphic sarcoma (12.7%), chondrosarcoma (6.6%), and well-differentiated/dedifferentiated liposarcoma (6.5%). The most commonly mutated genes were TP53 (47.5%), RB1 (9.8%), and ATRX (7.4%). The most common copy number variants were seen in MDM2 (11.4%), CDK4 (10.4%), CDKN2A (4.4%), CDKN2B (3.6%), and RB1 (3.0%). The most commonly altered pathways or classes of genes were cell cycle regulators, transcription factors, epigenetic regulators, and DNA damage response. A potentially clinically actionable mutation was seen in 58.6% of sarcomas sequenced. Germline variants were seen in the following genes, potentially suggesting a genetic predisposition to sarcoma: KIT mutation in 6.6% of the cohort, MET in 5.5%, PIK3CA in 3.9%, and ATM in 3.0%. These germline variants were seen across the spectrum of sarcoma subtypes.

Conclusion: This study suggests that routine clinical next generation sequencing of sarcomas has the potential to open additional treatment options for patients with sarcoma. It also suggests the possibility of certain germline variants predisposing to sarcoma. These findings may inform additional preclinical and clinical studies of specific genomic alterations in specific sarcoma subtypes. Future work will be needed to assess the clinical impact of using these data and whether these genetic alterations are as predictive of outcomes in sarcomas as they are in other cancers.



(P 401) OVEREXPRESSION OF CYCLIN DEPENDENT KINASES AND CYCLINS ACROSS SARCOMAS BEYOND CDK4

Daniel S. Lefler, MD[1]; Andrew Elliott, MD PhD[2]; Robert G. Maki, MD PhD[3]; Jaime F. Modiano, VMD PhD[4]; Andreas Seeber, MD PhD[5]; Richard F. Riedel, MD[6]; Nicholas D. Kendsersky, MD[7]; Aaron L. Sarver, PhD[4]; Andrea P. Espejo-Freire, MD[8]; Moh'd Khushman, MD[9]; Sosipatros A.

Boikos, MD[10]; Galina Lagos, MD[11]; Vaia Florou, MD MS[12]; Margaret von Mehren, MD[13]; Matthew Oberley, MD PhD[2]; Gina Z. D'Amato, MD[14]; Jonathan Trent, MD, PhD[15]; Atrayee Basu Mallick, MD[7], (1) University of Pennsylvania, Philadelphia, PA, (2) Caris Life Sciences, Phoenix, AZ, (3) Memorial Sloan Kettering Cancer Center; Professor, Weill Cornell Medical College, New York, NY, (4) University of Minnesota, Minneapolis, MN, (5) Medical University of Innsbruck, Innsbruck, Tirol, Austria, (6) Duke Cancer Institute, Durham, NC, (7) Thomas Jefferson University Hospital, Philadelphia, PA, (8) University of Minnesota, MInneapolis, MN, (9) Washington University in St. Louis / Siteman Cancer Center, St. Louis, MO, (10) Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, (11) Brown University / Lifespan Cancer Institute, Providence, RI, (12) Huntsman Cancer Institute University of Utah, SALT LAKE CITY, UT, (13) Fox Chase Cancer Center, Philadelphia, PA, (14) Sylvester Comprehensive Cancer Center, Miami, FL, (15) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL

Objective: Derangements in CDK4 and subsequent effects on the cell cycle have been described across sarcomas—most notably in liposarcoma (LPS), where the use of CDK4/6 inhibitors has been encouraging. However, other CDKs and their paired cyclins have important roles across malignancies. Consequently, inhibitors of CDK7, CDK9, and more are in development. We aimed to identify overexpressers of CDKs/cyclins across bone and soft tissue sarcomas in order to direct future investigation into their clinical relevance and novel therapeutic targeting.

Methods: DNA (592-gene or whole exome; N=7028) and RNA (whole transcriptome; N=3757) sequencing of 45 known genes encoding CDKs and cyclins was performed from sarcoma patient samples, representing 49 subtypes, submitted to Caris Life Sciences (Phoenix, AZ). High RNA expression of CDK/cyclin genes was defined as ≥ 1 and 2 standard deviations (SD) above the overall mean expression of each gene across all subtypes. We then identified the proportion of samples within each subtype that expressed CDK/cyclin genes ≥ 2 SD above the means, and we considered a threshold of ≥ 25% of samples significant for future evaluation. Samples were then evaluated for coincident expression of genes if the subtype-specific groups were found to overexpress multiple assessed genes.

Results: Multiple subtypes were found to have ≥ 25% of cases exhibiting overexpression of CDK/cyclin genes ≥ 2 SD above the grouped means per gene, as judged by RNA transcripts per million. Expected findings included CDK4 RNA overexpression by 145/224 (66%) cases of dedifferentiated LPS, 14/36 (39%) of well differentiated LPS, and 29/75 (39%) of LPS not otherwise specified. These correlated with amplifications of CDK4 in 65-86% of cases. Also expected was CCND1 overexpression in 28/97 (29%) cases of Ewing sarcoma, consistent with prior descriptions. Within rhabdomyosarcomas (RMS), 8/31 cases (26%) of alveolar RMS overexpressed CCND2, and 9/31 (29%) overexpressed CCNE1; 2 cases overexpressed both. 3/10 (30%) cases of spindle cell RMS overexpressed each of CDK6 and CDK8 (1 coincident), and 6/19 (32%) of embryonal RMS overexpressed CDK8. Chordoma was found to overexpress CDK7 in 33/78 (42%) of cases and CDK18 in 29/78 (37%) of cases; the majority of these (20) were coincident overexpressers. Clear cell sarcoma (CCS) overexpressed CDK2 (12/27, 44%) and CCNA1 (10/27, 37%); coincident overexpression was seen in 4 cases. Additionally, 10/37 (27%) of round cell sarcomas (RCS) overexpressed CCND2. Finally, 3/12 (25%) of intimal sarcomas overexpressed CDK4, 7/26 (27%) of epithelioid sarcomas overexpressed CCNA1, and 4/14 (29%) of PNETs overexpressed CCNB3. Although there were associations with a small number of other subtypes, there were fewer than 10 instances of each in the database, making interpretation difficult.

Conclusion: We identified overexpressers of CDK and cyclin genes among a heterogeneous sample of sarcoma patients. By setting a threshold of 2 SD above the mean of each gene we captured the top ~2% of expressers, and by considering subtypes with ≥ 25% of cases above that threshold we accurately identified known associations between CDK4/LPS and CCND1/Ewing sarcoma while also revealing new associations. The most notable overexpressers were chordoma (CDK7, CDK18), CCS (CDK2, CCNA1), RCS (CCND2), and RMS (CDK6, CDK8, CCND2, CCNE1). Associations between high expressers, other relevant mutations/fusions, and transcriptional signatures are under way. These mostly novel findings may be especially relevant in diseases where there no standard accepted therapies exist (e.g. chordoma, CCS). Future investigations may focus on the prognostic and predictive implications of these biomarkers (especially at different transcriptional thresholds), whether they drive tumorigenesis, and if relevant therapeutic targeting yields clinical benefit.

(P 402) TISSUE PERFUSION AND WOUND HEALING AFTER SOFT TISSUE SARCOMA RESECTION

Precious C. Oyem, BA[1]; Zachary D. Burke, MD[2]; Nathan W. Mesko, MD[1]; Lukas M. Nystrom, MD[1], (1) Cleveland Clinic, Cleveland, OH, (2) Celveland Clinic Sarcoma Program, Cleveland, OH

Objective: Soft tissue sarcoma is a rare but potentially lethal malignancy with an incidence of 12,750 in 2019 alone. Because of the complex nature of these tumors, surgical resection in combination with radiation therapy is the mainstay of treatment. While the benefits of this dual treatment have been proven, radiation also has well-described negative consequences on long-term tissue integrity and wound healing. An estimated 35% of all patients that receive preoperative radiation experience wound complications. However, outside the known comorbidities such as smoking and diabetes that impair wound healing, there are no reliable clinical criteria that can predict wound complications in patients. It has been suggested that wound healing potential in sarcoma can be evaluated using intraoperative indocyanine green fluorophore (ICG) fluorescence imaging to assess perfusion. Therefore, we aim to investigate the association between tissue perfusion and wound outcomes after resection of soft tissue sarcoma. This is a preliminary report of an investigation that is ongoing.

Methods: This is a prospective clinical study. Patients with biopsy-proven soft tissue sarcoma who were to be treated with limb-sparing surgical excision and primary skin closure were prospectively enrolled. Utilizing Near-infrared fluorescent imaging (NIRF), a video sequence of intravenous ICG was recorded intraoperatively. These videos were recorded at 3 points intraoperatively: before tumor resection, after resection, and after wound closure. From these videos, perfusion-time graphs were generated which correlate fluorescence intensity to tissue perfusion. Patients are then followed for 6 months postoperatively to assess for wound complications. The postoperative wound during follow-up in every patient was compared to the perfusion curves generated intraoperatively. Wound complication was the primary outcome of interest which was subdivided into: drainage/delayed healing, necrosis, dehiscence, seroma,

hematoma, superficial and deep infection, each of these with its own annotation.

Results: To date, 25 of the planned 40 patients have been enrolled and 18 have been analyzed. Most tumors were located in the thigh (61.1%). Myxofibrosarcoma (27.8 %) was the most common histology. 15 patients (83.3%) received preoperative radiation. 10 patients (55.6%) experienced wound complications with 5 (50 %) requiring return to OR (Table 1). While some patients' perfusion-time curves showed a clear distinction between the different annotations (Figure 1), this was not consistent across every patient who experienced wound complications. We also observed some differences in the parameter mapping showing ICG ingress and area under the curve (AUC). Data collection and analysis are ongoing.

Conclusion: Intraoperative assessment of tissue perfusion may provide predictive insight into wound healing potential. This investigation is ongoing.

Table 1: Demographic and Patient Characteristics stratified by the Presence or Absence of Wound Complications

	Wound Complication (N=10)	No Wound Complications (N=8)	Total (N=18)	p value
Age, Years (Mean (SD))	60.60 (12.64)	56.25 (12.69)	58.67 (12.49)	0.479
Sex				1
Female	5 (50.0%)	4 (50.0%)	9 (50.0%)	
Male	5 (50.0%)	4 (50.0%)	9 (50.0%)	
Race				**< 0.001**
African American	0 (0.0%)	6 (75.0%)	6 (33.3%)	
Caucasian	10 (100.0%)	2 (25.0%)	12 (66.7%)	
BMI	30.63 (7.41)	32.94 (11.37)	31.66 (9.15)	0.611
Location				0.407
Knee area	0 (0.0%)	1 (12.5%)	1 (5.6%)	
Pelvis/Hip area	2 (20.0%)	2 (25.0%)	4 (22.2%)	
Shoulder girdle	2 (20.0%)	0 (0.0%)	2 (11.1%)	
Thigh	6 (60.0%)	5 (62.5%)	11 (61.1%)	
Histology				0.251
Dedifferentiated Liposarcoma	2 (20.0%)	0 (0.0%)	2 (11.1%)	
MPNST	1 (10.0%)	0 (0.0%)	1 (5.6%)	
Myxofibrosarcoma	4 (40.0%)	1 (12.5%)	5 (27.8%)	
Myxoid Liposarcoma	1 (10.0%)	3 (37.5%)	4 (22.2%)	
Solitary fibrous tumor	0 (0.0%)	1 (12.5%)	1 (5.6%)	
Spindle cell sarcoma	0 (0.0%)	1 (12.5%)	1 (5.6%)	
Synovial Sarcoma	1 (10.0%)	0 (0.0%)	1 (5.6%)	
Undifferentiated Pleomorphic Sarcoma	1 (10.0%)	2 (25.0%)	3 (16.7%)	
Grade 3 tier				0.645
Low (1)	4 (40.0%)	2 (25.0%)	6 (33.3%)	
Intermediate (2)	3 (30.0%)	2 (25.0%)	5 (27.8%)	
High (3)	3 (30.0%)	3 (37.5%)	6 (33.3%)	
Unknown	0 (0.0%)	1 (12.5%)	1 (5.6%)	
Radiotherapy Given	9 (90.0%)	6 (75.0%)	15 (83.3%)	0.396
Preoperative Radiotherapy	9 (90.0%)	6 (75.0%)	15 (83.3%)	0.396
Reoperation	5 (50.0%)	1 (12.5%)	6 (33.3%)	0.094

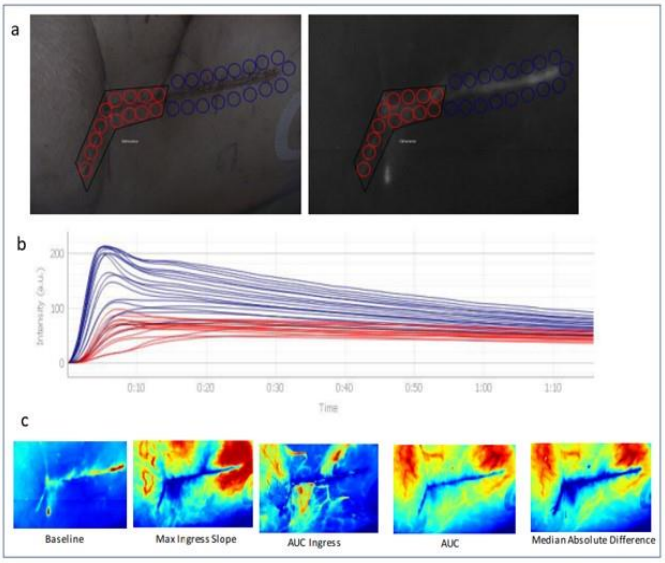


Figure 1: (a) A patient who experienced wound dehiscence at the proximal half of incision (black outline, red markings); (b) the corresponding perfusion curve (red=dehisced region; blue=normal region); (c) Parameter mapping showing baseline, ingress, and AUC.

(P 403) THE IMPACT OF PATIENT, TUMOR, AND TREATMENT FACTORS ON SURVIVAL AND METASTASIS OUTCOMES IN PATIENTS WITH MYXOID LIPOSARCOMA

Yasser Abusabha, BS[1]; James McMullen, BA[2]; Mihir Surapaneni, BBA[2]; Geoffrey Siegel, MD[3], (1) University of Michigan Medical School, Canton, MI, (2) University of Michigan Medical School, Ann Arbor, MI, (3) University of Michigan Department of Orthopedic Surgery, Ann Arbor, MI

Objective: Myxoid liposarcomas are rare soft tissue tumors which pose challenges to physicians attempting to develop treatment protocols or deliver prognostications to patients. Current literature on myxoid liposarcoma has noted a few prognostic factors such as tumor size and grade; however, optimal therapeutic strategies, including the role of chemoradiotherapy, remain debated. This retrospective study seeks to identify patient and tumor factors associated with disease-free, metastasis-free, and overall survival rates in patients with myxoid liposarcoma. Additionally, we evaluated the efficacy of different treatment modalities and their association with patient outcomes. By identifying factors that influence prognosis, this study may contribute to better patient education, informed decision-making, and the development of personalized treatment strategies to improve outcomes in patients affected by myxoid liposarcoma.

Methods: A retrospective cohort study was done using data from a large academic institution's sarcoma archives. Patients diagnosed with myxoid liposarcoma between January 1, 1997, and

June 24, 2021, were considered for this study. Patients over 18 years of age with histopathologic confirmation of myxoid liposarcoma and no known metastases at the time of presentation were identified for further review. Patient demographics, tumor characteristics, treatment(s) received, and outcomes were collected from electronic medical records. Statistical analyses were performed to assess the impact of various factors on disease-specific survival and metastasis-free rates at different time endpoints.

Results: Our cohort of 66 patients had a 15-year disease-specific survival rate of 73%. In addition, 56% of our cohort remained metastasis free at the same time endpoint. Demographic factors such as age, sex, race, body mass index (BMI), and smoking history did not demonstrate a significant association with survival outcomes. Tumor grade and volume also did not show a significant impact on survival rates. Patients who underwent primary tumor resection at our tertiary center had improved survival compared to those treated at outside hospitals. Notably, neoadjuvant chemotherapy and adjuvant radiotherapy demonstrated favorable outcomes compared to other treatment approaches. Moreover, patients who received combined chemoradiotherapy had significantly better outcomes compared to patients who received monotherapy.

Conclusion: This study contributes to the understanding of myxoid liposarcoma by identifying potential prognostic factors and treatment modalities that can enhance patient outcomes. The findings suggest that chemotherapy and adjuvant radiotherapy may improve survival rates in myxoid liposarcoma patients. However, further research is needed to validate these results and explore additional molecular markers and genetic abnormalities that may influence prognosis. Nonetheless, this study provides valuable evidence for the development of personalized treatment strategies to improve outcomes in myxoid liposarcoma.

Table 1 Descriptive Statistics

	n = 66 (100%)
Demographics	
Age (years)	
< 45	29 (44%)
≥ 45	37 (56%)
Average: 48 years	
Sex	
Female	30 (45%)
Male	36 (55%)
Race	
Black	7 (11%)
White	53 (80%)
Other or Unknown	6 (9%)
BMI	
< 25 kg/m²	12 (18%)
25 kg/m² – 30 kg/m²	20 (30%)
≥ 30 kg/m²	34 (52%)
Average: 30.9 kg/m²	
Smoking History	
Current or Former	27 (41%)
Never-Smoker	39 (59%)
Tumor Characteristics	
Grade	
Low (1)	16 (21%)
High (2-3)	50 (76%)
Unknown	2 (3%)
Tumor Volume	
< 475 cm³	34 (52%)
≥ 475 cm³	32 (48%)
Median: 475 cm³	
Average: 840 cm³	
Primary Tumor Location	
Lower Extremity	61 (92%)
Non-Extremity	5 (8%)
Treatments	
Surgery Location	
Home Institution	33 (50%)
Outside Hospital	33 (50%)
Surgery	
Wide Resection	64 (97%)
Amputation	2 (3%)
Chemotherapy	
Neoadjuvant	16 (24%)
Adjuvant	20 (30%)
Radiation Therapy	
Neoadjuvant	18 (27%)
Adjuvant	42 (64%)
Chemoradiotherapy	
None	5 (8%)
Monotherapy	29 (44%)
Combination Therapy	32 (48%)
Outcomes	
Local Recurrence	
Yes	11 (17%)
Average Time to Recurrence (since primary treatment): 7.2 years	
Metastasis	
Yes	30 (45%)
Average Time to Metastasis (since primary treatment): 4.9 years	
Death (secondary to disease)	
Yes	20 (30%)
Disease Related Mortality: 30%	
Time to Death (since first presentation)	
< 5 years	7 (35% of patients who died)
> 5 years	13 (65% of patients who died)
Average: 8.9 years	

Abbreviation: BMI, Body Mass Index

Table II Patient Demographics, Tumor Characteristics, and Treatment Modalities with Disease-Specific Survival Rates

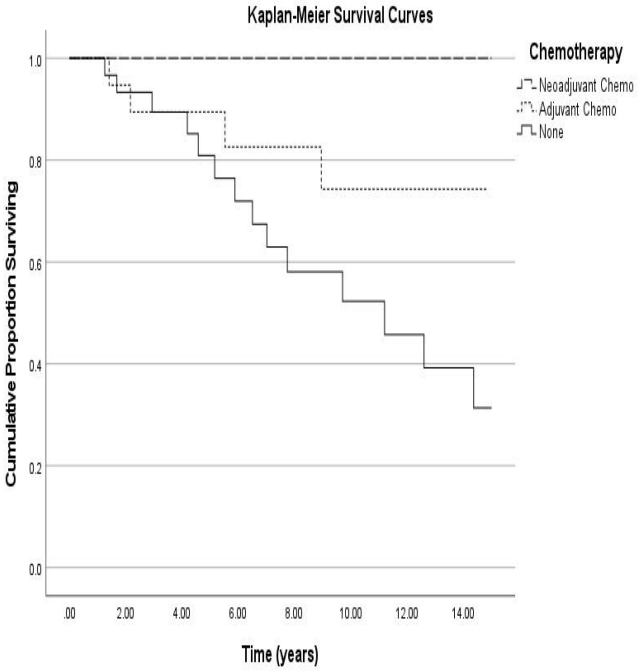
	2-year survival rate	5-year survival rate	7-year survival rate	10-year survival rate	12-year survival rate	15-year survival rate
Overall (n = 66)						
	97%	89%	85%	79%	76%	73%
Age (years)						
< 45	100%	86%	83%	83%	79%	79%
≥ 45	95%	92%	87%	76%	73%	68%
	p = 0.20	p = 0.49	p = 0.68	p = 0.68	p = 0.78	p = 0.54
Sex						
Male	94%	83%	81%	72%	67%	64%
Female	100%	97%	90%	87%	87%	83%
	p = 0.21	p = 0.10	p = 0.36	p = 0.25	p = 0.12	p = 0.13
Race						
Black	100%	71%	57%	57%	57%	57%
White	96%	91%	87%	79%	76%	72%
Other	100%	100%	100%	100%	100%	100%
Unknown	100%	100%	100%	100%	100%	100%
	p = 0.92	p = 0.49	p = 0.23	p = 0.45	p = 0.53	p = 0.54
Body Mass Index (BMI)						
< 25 kg/m²	100%	100%	92%	92%	92%	92%
≥ 25 kg/m²	96%	87%	83%	76%	72%	69%
	p = 0.52	p = 0.20	p = 0.43	p = 0.23	p = 0.17	p = 0.12
Smoking History						
Current or Former	96%	93%	85%	82%	78%	74%
Never-Smoker	97%	87%	85%	77%	74%	72%
	p = 0.77	p = 0.49	p = 0.92	p = 0.64	p = 0.70	p = 0.79
Primary Tumor Resection Location						
Home Institution	97%	82%	76%	88%	88%	82%
Outside Hospital	97%	97%	94%	70%	64%	64%
	p = 1.00	p = 0.06	p = 0.06	p = 0.11	p = 0.05	p = 0.16
Tumor Grade						
Low (1)	100%	100%	79%	64%	57%	50%
High (2-3)	96%	86%	86%	82%	82%	80%
	p = 0.47	p = 0.16	p = 0.61	p = 0.26	p = 0.12	p = 0.10
Tumor Volume						
< 475 cm³	97%	91%	91%	85%	82%	82%
≥ 475 cm³	97%	88%	79%	73%	70%	64%
	p = 0.96	p = 0.74	p = 0.24	p = 0.29	p = 0.35	p = 0.26
Primary Tumor Location						
Extremity	97%	92%	87%	80%	77%	74%
Non-Extremity	100%	60%	60%	60%	60%	60%
	p = 0.68	p = 0.03	p = 0.13	p = 0.31	p = 0.38	p = 0.51
Chemotherapy						
None	97%	84%	74%	68%	61%	55%
Neoadjuvant	100%	100%	100%	100%	100%	100%
Adjuvant	95%	90%	90%	80%	80%	80%
	p = 0.70	p = 0.25	p = 0.05	p = 0.05	p = 0.02	p = 0.01
Radiotherapy						
None	100%	88%	88%	75%	63%	50%
Neoadjuvant	88%	82%	71%	65%	59%	59%
Adjuvant	100%	93%	90%	85%	85%	83%
	p = 0.06	p = 0.28	p = 0.02	p = 0.01	p < 0.001	p < 0.001
Chemoradiotherapy						
None	100%	80%	80%	80%	60%	40%
Monotherapy	97%	86%	76%	66%	62%	59%
Combination	97%	94%	94%	91%	91%	91%
	p = 0.93	p = 0.49	p = 0.14	p = 0.05	p = 0.03	p = 0.01

Note: p value shown for Log-rank test between all variables.

Table III Patient Demographics, Tumor Characteristics, and Treatment Modalities with Metastasis-Free Rates

	2-year metastasis-free rate	5-year metastasis-free rate	7-year metastasis-free rate	10-year metastasis-free rate	12-year metastasis-free rate	15-year metastasis-free rate
Overall (n = 66)						
	86%	74%	68%	61%	59%	56%
Age (years)						
< 45	79%	69%	69%	62%	62%	59%
≥ 45	92%	78%	68%	60%	57%	54%
	p = 0.14	p = 0.38	p = 0.98	p = 0.94	p = 0.80	p = 0.78
Sex						
Male	81%	69%	64%	58%	56%	53%
Female	93%	80%	73%	63%	63%	60%
	p = 0.15	p = 0.37	p = 0.51	p = 0.85	p = 0.74	p = 0.79
Race						
Black	71%	57%	43%	43%	43%	43%
White	89%	76%	70%	62%	60%	57%
Other	100%	100%	100%	100%	100%	100%
Unknown	67%	67%	67%	33%	33%	33%
	p = 0.37	p = 0.56	p = 0.36	p = 0.31	p = 0.33	p = 0.37
Body Mass Index (BMI)						
< 25 kg/m²	100%	92%	92%	75%	75%	75%
≥ 25 kg/m²	83%	70%	63%	57%	56%	52%
	p = 0.14	p = 0.14	p = 0.06	p = 0.20	p = 0.18	p = 0.15
Smoking History						
Current or Former	89%	82%	74%	56%	56%	52%
Never-Smoker	85%	69%	64%	64%	62%	59%
	p = 0.59	p = 0.25	p = 0.37	p = 0.74	p = 0.84	p = 0.73
Primary Tumor Resection Location						
Home Institution	94%	91%	82%	79%	76%	73%
Outside Hospital	79%	58%	55%	42%	42%	39%
	p = 0.07	p = 0.003	p = 0.02	p = 0.005	p = 0.01	p = 0.01
Tumor Grade						
Low (1)	100%	71%	57%	36%	29%	29%
High (2-3)	82%	76%	72%	70%	70%	66%
	p = 0.10	p = 0.80	p = 0.38	p = 0.05	p = 0.02	p = 0.03
Tumor Volume						
< 475 cm³	88%	76%	73%	70%	70%	70%
≥ 475 cm³	85%	73%	64%	52%	49%	42%
	p = 0.73	p = 0.80	p = 0.51	p = 0.22	p = 0.17	p = 0.08
Primary Tumor Location						
Extremity	89%	75%	69%	62%	61%	57%
Non-Extremity	60%	60%	60%	40%	40%	40%
	p = 0.10	p = 0.45	p = 0.70	p = 0.47	p = 0.47	p = 0.47
Chemotherapy						
None	77%	58%	52%	39%	36%	32%
Neoadjuvant	100%	100%	100%	100%	100%	93%
Adjuvant	90%	80%	70%	65%	65%	65%
	p = 0.10	p = 0.01	p = 0.004	p < 0.001	p < 0.001	p < 0.001
Radiotherapy						
None	88%	75%	75%	63%	63%	50%
Neoadjuvant	70%	41%	41%	41%	41%	35%
Adjuvant	93%	88%	78%	68%	66%	66%
	p = 0.07	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001
Chemoradiotherapy						
None	80%	80%	80%	60%	60%	40%
Monotherapy	79%	55%	48%	38%	35%	35%
Combination	94%	91%	84%	81%	81%	78%
	p = 0.24	p = 0.01	p = 0.01	p = 0.001	p < 0.001	p < 0.001

Note: p value shown for Log-rank test between all variables.



(P 404) PROTEOMIC PROFILING OF THE SOFT TISSUE SARCOMA MICROENVIRONMENT IDENTIFIES A PROGNOSTIC SIGNATURE BASED ON PROTEOGLYCAN EXPRESSION AND CD8+ TUMOUR INFILTRATING LYMPHOCYTES

Valeriya Pankova, Msc[1]; Jessica Burns, PhD[2]; Khin Thway, MD[3]; Robin L. Jones, BSc, MB BS, MRCP, MD[4]; Paul Huang, PhD[2], (1) The Institute of Cancer Research, Sutton, England, United Kingdom, (2) The Institute of Cancer Research, London, England, United Kingdom, (3) The Royal Marsden NHS Foundation Trust, London, England, United Kingdom, (4) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom

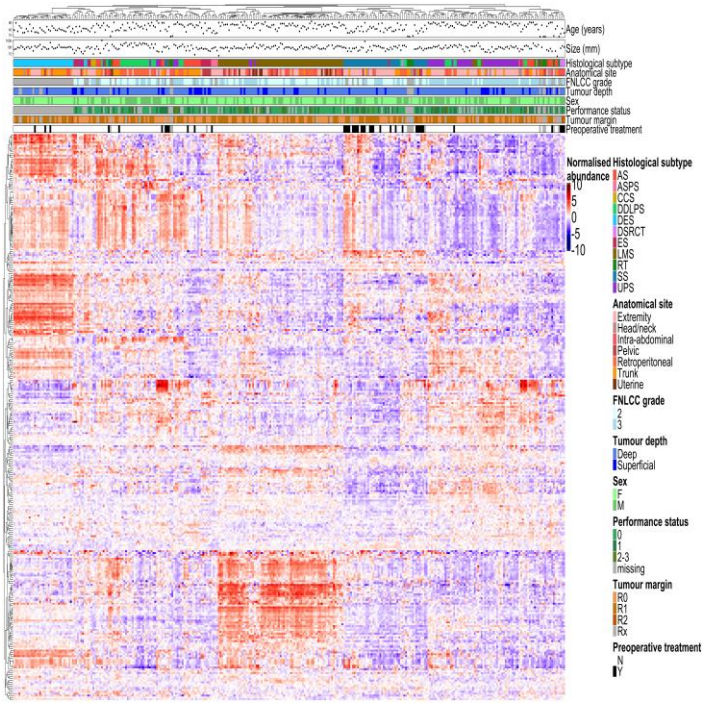
Objective: Components of the tumour microenvironment (TME), such as the extracellular matrix (ECM) and their cognate adhesion receptors, drive important hallmarks of tumorigenesis, including tumour progression and metastasis. These components remain poorly characterised in soft tissue sarcomas (STS), and there is a limited understanding of their role in STS pathobiology. Here, we undertook a comprehensive proteomic characterisation of the STS TME in a large cohort of patients to identify candidate prognostic biomarkers for risk stratification.

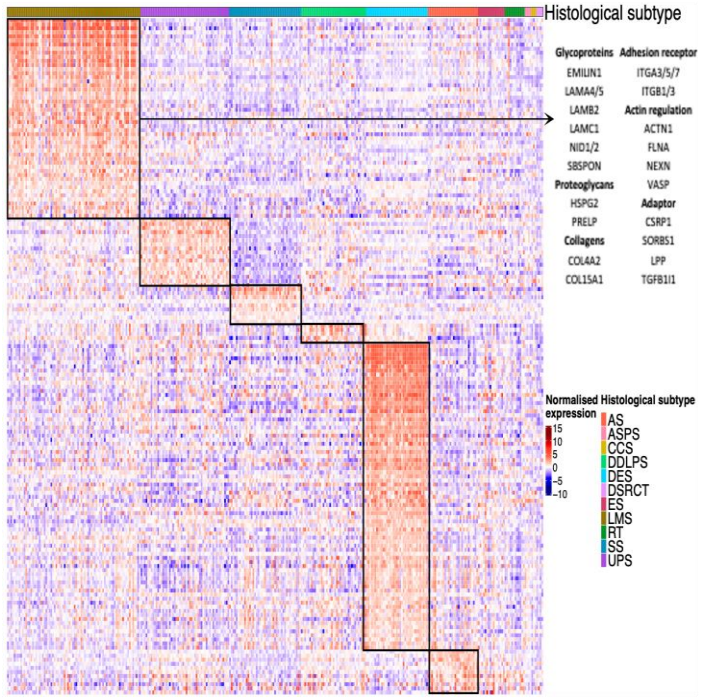
Methods: We employed mass spectrometry to profile the STS TME (including the ECM and their cognate adhesion receptors) in primary tumour specimens from a cohort of 321 STS patients across 11 histological subtypes. This led to the identification of 302 ECM related proteins. Significance Analysis of Microarrays Two-class unpaired tests were utilised to define TME proteins significantly enriched in each histological subtype. To investigate the intra-subtype tumour heterogeneity of the TME within leiomyosarcomas (LMS) (n=80) and

dedifferentiated liposarcomas (DDLPS) (n=39), consensus clustering of ECM related proteomic profiles was performed. Univariate Cox regression analysis of single-sample Gene Set Enrichment Analysis (ssGSEA) scores of different TME gene subsets was evaluated to identify candidate prognostic protein signatures. In parallel, tumour infiltrating lymphocyte (TIL) levels (CD3, CD4 and CD8) were assessed by immunohistochemistry in LMS, DDLPS and undifferentiated pleomorphic sarcoma (UPS) (n=148) cases. Multivariable Cox regression analysis was performed to determine whether the ssGSEA prognostic signatures were independent of known prognostic clinicopathological variables.

Results: Unsupervised clustering of the dataset demonstrated that distinct histological subtypes had unique TME proteomic profiles (Figure 1A). Supervised analysis of significantly enriched proteins showed that LMS tumours had upregulated levels of proteins involved in cell adhesion (e.g. ITGA3/5/7 and ITGB1/3) and various glycoproteins (e.g. LAMA4/5, LAMB2, LAMC1, NID1/2, SBSPON) (Figure 1B). LMS and DDLPS showed intra-subtype heterogeneity of TME protein expression with consensus clustering identifying three significant subgroups of patients in each subtype. In the case of LMS, these subgroups were associated with tumour grade, while in DDLPS, the subgroups were prognostic of local recurrence-free survival and overall survival (OS). In a subset of LMS, UPS and DDLSP patients (n=148), integrating a ssGSEA score of 11 proteoglycans together and CD8+ TILs count was prognostic for OS (Figure 1C). Patients with low proteoglycan score/low CD8+ TILs had a significantly worse OS compared to patients with a high proteoglycan score/high CD8+ TILs (univariate analysis: Hazard Ratio (HR)=0.33, 95% Confidence Interval (CI)=0.17-0.65, p-value=0.001). Moreover, after adjusting for tumour grade, size, histological subtype, performance status, sex and age, low proteoglycan score/low CD8+ TILs score remained an independent prognostic factor for OS (multivariable analysis: HR=0.33, 95% CI=0.16-0.70, p-value=0.004).

Conclusion: Our characterisation of the TME in a large cohort of STS patients showed that STS histological subtypes exhibit unique TME protein expression profiles. There is intra-subtype TME heterogeneity in LMS and DDLPS, which is associated with tumour grade and survival outcome measures. Integration of the proteoglycan score with CD8+ TILs counts led to the discovery of an independent prognostic factor for OS in LMS, DDLPS and UPS patients.


Age (years)
Size (mm)
Histological subtype
Anatomical site
FNLCC grade
Tumour depth
Sex
Performance status
Tumour margin
Preoperative treatment
Normalised abundance
10
5
0
-5
-10
Histological subtype
AS
ASPS
CCS
DDLPS
DES
DSRCT
ES
LMS
RT
SS
UPS
Anatomical site
Extremity
Head/neck
Intra-abdominal
Pelvic
Retroperitoneal
Trunk
Uterine
FNLCC grade
2
3
Tumour depth
Deep
Superficial
Sex
F
M
Performance status
0
1
2-3
missing
Tumour margin
R0
R1
R2
Rx
Preoperative treatment
N
Y


Histological subtype
Glycoproteins
EMILIN1
LAMA4/5
LAMB2
LAMC1
NID1/2
SBSPON
Proteoglycans
HSPG2
PRELP
Collagens
COL4A2
COL15A1
Adhesion receptor
ITGA3/5/7
ITGB1/3
Actin regulation
ACTN1
FLNA
NEXN
VASP
Adaptor
CSRP1
SORBS1
LPP
TGFB1I1
Normalised expression
15
10
5
0
−5
−10
Histological subtype
AS
ASPS
CCS
DDLPS
DES
DSRCT
ES
LMS
RT
SS
UPS

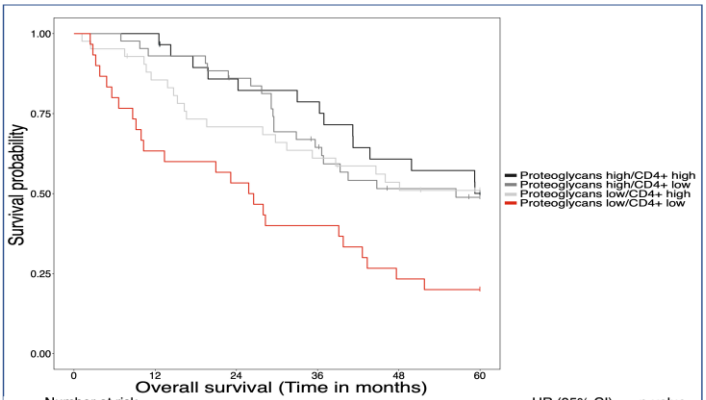


Figure 1: A) Annotated heatmap illustrating the unsupervised clustering (Pearson's correlation distance) of TME proteins, including the ECM and their cognate adhesion receptor proteins in the STS cohort. Annotation panels at the top correspond to patient age, tumour size, histological subtype, anatomical site, tumour grade, patient sex, performance status, surgical margin and preoperative treatment status. Abbreviations: AS = angiosarcoma; ASPS = alveolar soft part sarcoma; CCS = clear cell sarcoma; DDLPS = dedifferentiated liposarcoma; DES = desmoid tumour; DSRCT = desmoplastic small round cell tumour; EPS =epithelioid sarcoma; LMS = leiomyosarcoma; RT = rhabdoid tumour; SS = synovial sarcoma; UPS=undifferentiated pleomorphic sarcoma; R = residual tumour; Y = yes; N = no. B) Heatmap showing TME proteins uniquely upregulated (indicated by black boxes) in histological subtypes (false discovery rate < 0.01, fold change ≥ 2), arranged by histological subtype. A selection of proteins which are upregulated in LMS are highlighted. C) Kaplan Meier plot of overall survival with stratification by proteoglycan score and CD4+ TIL count. Hazard ratio (HR), 95% confidence intervals (CI) and p-values were determined by univariate cox regression with two-sided Wald test.

(P 405) **PRIMARY CARDIAC ANGIOSARCOMA- GENOMIC AND MOLECULAR LANDSCAPE**

Aparna Subramaniam, MBBS, MPH[1]; Chia-Chin wu, PhD[1]; Hannah Beird, PhD[2]; Jianhua Zhang, PhD[1]; Alexander J. Lazar, MD, PhD[1]; Davis R. Ingram, BS[1]; Khalida Wani, PhD[1]; Alexa Bello, MD[1]; Areeba Ali, MD[3]; Marvin Atkins, MD[3]; Robert Benjamin, MD[1]; Shreyaskumar Patel, MD[1]; Michael J. Reardon, MD[3]; P Andrew Futreal, PhD[1]; Vinod Ravi, MD, MS, MBA[1], (1) The University of Texas MD Anderson Cancer Center, Houston, TX, (2) Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA, Houston, TX, (3) Houston Methodist Research Institute, Houston, TX

Objective: Primary cardiac angiosarcoma (CAS) is the most common histological type of primary cardiac sarcomas. The most common site of tumor is the right atrium of the heart. The median overall survival with surgical resection ranges from 8-9 months.
The genomic and molecular characteristics of primary CAS are poorly understood with only 3 families with cases of CAS that had exome or POT1 sequencing performed in one study on TP53 negative Li-Fraumeni like families and two CAS patients with exome profiling in the Angiosarcoma Count-Me-In project. Understanding the genomic landscape of primary CAS can be valuable in developing novel therapies and improving survival outcomes for patients.

Methods: Patients with a diagnosis of primary CAS seen at MD Anderson Cancer Center and Houston Methodist Research Institute between 1/1/2001 and 12/31/2015 were identified using an electronic database system. The tissue bank database was searched for availability of tumor tissue samples for these patients. Whole exome sequencing (WES) was performed on 20 primary CAS samples obtained from 13 unique patients (17 frozen, 3 formalin-fixed paraffin

embedded). SNP array was performed on 15 samples and RNA sequencing was performed on 3 samples. The reads of WES were mapped to the hg19 reference genome using BWA-MEM, and MuTect was used to call somatic mutations. The DNAcopy R package was used to analyze copy number data derived from SNP array. RNA sequencing reads of the samples were mapped to the hg19 reference genome using the STAR aligner. For calculation of gene expression, raw count data of each gene were first obtained using HTSeq and normalized to TPM (Transcripts Per Million). Finally, we used a rank-based method, single-sample gene set enrichment scores (ssGSEA), to compare the gene expression data of two batches of our samples. Immune infiltration scores of samples across different cancer types were calculated from the gene expression data using ESTIMATE. Clinical data was abstracted manually from the electronic medical records.

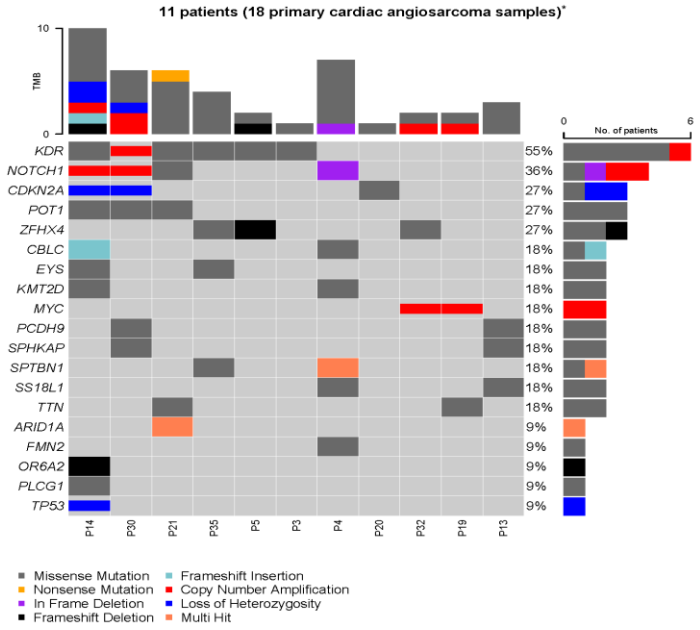
Results: The cohort was predominantly male (77%) with a median age of diagnosis of 44 years. The right atrium was the most common site of tumor (n= 11 patients) and 8 patients had metastatic disease at the time of diagnosis (Table 1). The median overall survival was 21.8 months.

We identified 1706 nonsynonymous point mutations with an average of 58.6 mutations/ sample and a median tumor mutation burden of 1.45 mut/Mb. The most frequently mutated genes were KDR, POT1, CDKN2A, NOTCH1 and ZFHX4. 5 of the missense mutations in KDR (4 encoding p.Thr771Arg and 1 encoding p.Thr771Lys) are located in the transmembrane domain. Copy number amplification of KDR was detected in 1 patient. The mutations in POT1 (2 encoding p.Ala230Val, 2 encoding p.Ile220Ser and 1 encoding p.Tyr26Cys) occurred in the portion encoding the OB1 and OB2 region of the protein that makes direct contact with DNA. A germline variant in POT1 p.Arg117His was also detected in 1 patient. Co-occurrence of KDR and POT1 mutations were seen in 2 patients. 4 alterations were detected in NOTCH1 including 1 missense mutation (encoding p.Cys461Trp), 1 in frame deletion and copy number amplifications in 2 patients. 2 patients had a loss of heterozygosity in CDKN2A, while 1 patient had a missense mutation (encoding p.Ala21Val). 1 patient had a mutation in PLCG1 (encoding p.Arg707Gln) (Figure 1). We did not detect any focal copy number amplifications or deletions by SNP array analysis. Gains spanned chromosomes 7 and 8, with RECQL and SLC39A4 being the frequently amplified cancer genes on chromosome 8.

Primary CAS was not significantly different from AS of other sites on performing ssGSEA using RNAseq data from 3 samples. The ESTIMATE immune score for these samples were also high.

Conclusion: Our results highlight the presence of KDR mutations in primary CAS that have been reported predominantly in primary breast AS in previous studies. Primary CAS is characterized by POT1 mutations, which have previously been reported only in CAS in Li-Fraumeni like families. We are limited by the small number of samples with RNAseq data to derive inferences about the immune profile of CAS, which is also confounded by chemotherapy administered to the patients prior to surgical resection. Functional assays to understand the effect of mutations in the transmembrane domain of KDR are needed to determine if it is a potential therapeutic target.

ID	Gender	Tumor site	Age at diagnosis	Stage at diagnosis	Sites of metastases	Treatments
P3	Female	Right atrium	51	Metastatic	Brain, Bone, Lungs	Adriamycin + Ifosfamide > Gemcitabine + Docetaxel > Surgery
P4	Male	Main pulmonary artery	44	Localized	Left Pulmonary Artery	Adriamycin + Dacarbazine > Gemcitabine + Docetaxel > Surgery > Gemcitabine + Docetaxel > Adriamycin + Ifosfamide
P5	Female	Right atrium	18	Locally advanced	Liver	Paclitaxel > Adriamycin + Ifosfamide > Surgery > Adriamycin + Ifosfamide > Radiation
P13	Male	Right atrium	29	Metastatic	Bone	Paclitaxel > Radiation > Adriamycin + Ifosfamide > Surgery
P14	Male	Right atrium	44	Localized	Bone, Mediastinum, Liver	Adriamycin + Ifosfamide > Docetaxel > Ifosfamide > Surgery
P19	Male	Right atrium	58	Localized	None	Adriamycin + Ifosfamide > Gemcitabine + Docetaxel > Gemcitabine > Paclitaxel > Surgery
P20	Male	Right atrium	30	Metastatic	Bone	Adriamycin + Ifosfamide > Surgery
P21	Male	Right atrium	57	Metastatic	Lungs, Pleura, Liver	Adriamycin + Ifosfamide > Gemcitabine + Docetaxel > Gemcitabine > Paclitaxel > Surgery > c-Met inhibitor > Gemcitabine + Docetaxel > Adriamycin + Ifosfamide > Gemcitabine + Pazopanib > Paclitaxel
P30	Male	Right atrium	46	Metastatic	Liver	Adriamycin + Ifosfamide > Gemcitabine + Docetaxel > Surgery > Gemcitabine + Docetaxel > Pazopanib > Bevacizumab + Paclitaxel > Sorafenib
P32	Male	Right ventricle	61	Metastatic	Lungs	Adriamycin + Ifosfamide > Gemcitabine + Docetaxel > Surgery > Paclitaxel
P33	Female	Right atrium	42	Localized	None	Adriamycin + Ifosfamide > Surgery > Adriamycin + Ifosfamide > Gemcitabine + Docetaxel > Paclitaxel
P35	Male	Right atrium	40	Metastatic	Lungs	Adriamycin + Ifosfamide > Gemcitabine + Docetaxel > Surgery
P36	Male	Right atrium	21	Metastatic	Lungs, Bone	Gemcitabine + Paclitaxel > Adriamycin + Ifosfamide > Surgery> Gemcitabine + Paclitaxel > Pazopanib



(P 406) EXTENSIVE ANALYSIS OF 59 SARCOMA-RELATED FUSION GENES IDENTIFIED PAZOPANIB AS A POTENTIAL INHIBITOR TO COL1A1-PDGFB FUSION GENE

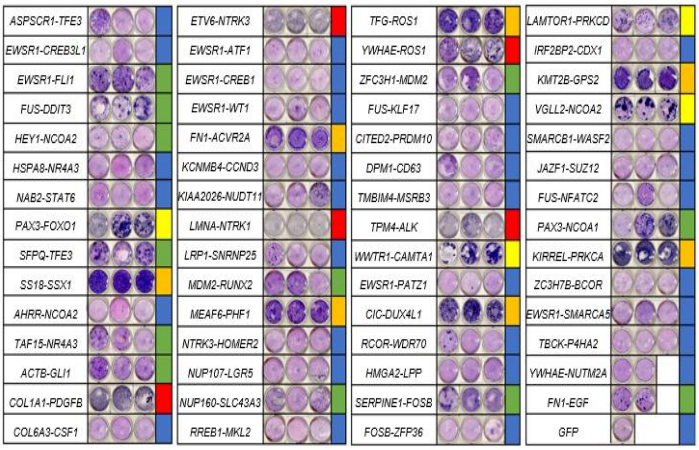
Takeshi Hirose, MD/PhD[1]; Masachika Ikegami, MD/PhD[2]; Shinya Kojima, Researcher[2]; Akihiko Yoshida, MD, Ph.D[3]; Makoto Endo, MD, PhD[4]; Eijiro Shimada, MD/PhD[5]; Masaya Kanahori, MD[1]; Ryunosuke Oyama, MD[1]; Yoshihiro Matsumoto, N/A, MD/PhD[1]; Yasuharu Nakashima, MD/PhD[1]; Akira Kawai, MD, Ph.D[6]; Hiroyuki Mano, MD/PhD[7]; Shinji Kohsaka, MD/PhD[2], (1) Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Fukuoka, Japan, (2) Division of Cellular Signaling, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan, (3) Department of Diagnostic Pathology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (4) Kyushu University, Fukuoka, Fukuoka, Japan, (5) Duke University, Chapel Hill, NC, (6) National Cancer Center Hospital, Tokyo, Tokyo, Japan, (7) National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan

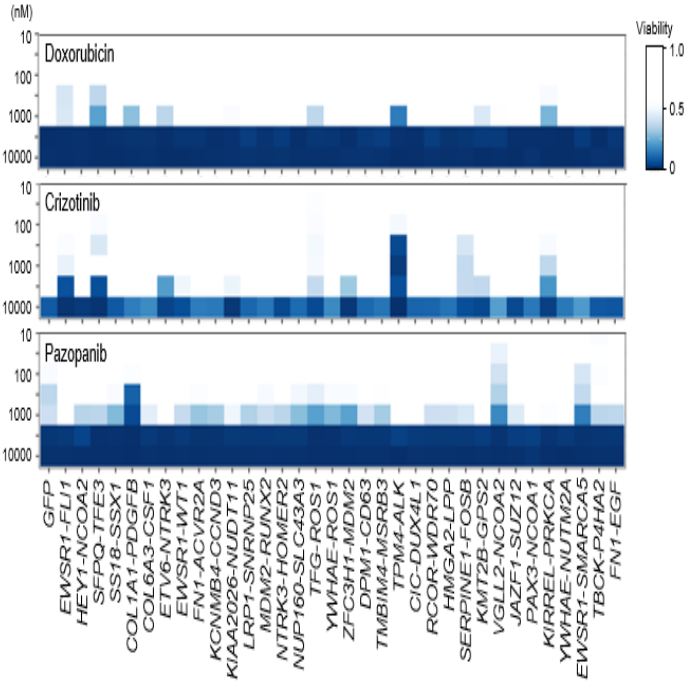
Objective: Sarcomas are malignant mesenchymal tumors that are extremely rare and divergent. Fusion genes are involved in approximately 30% of sarcomas as driver oncogenes; however, their detailed functions are not fully understood. In this study, we determined the functional significance of 59 sarcoma-related fusion genes.

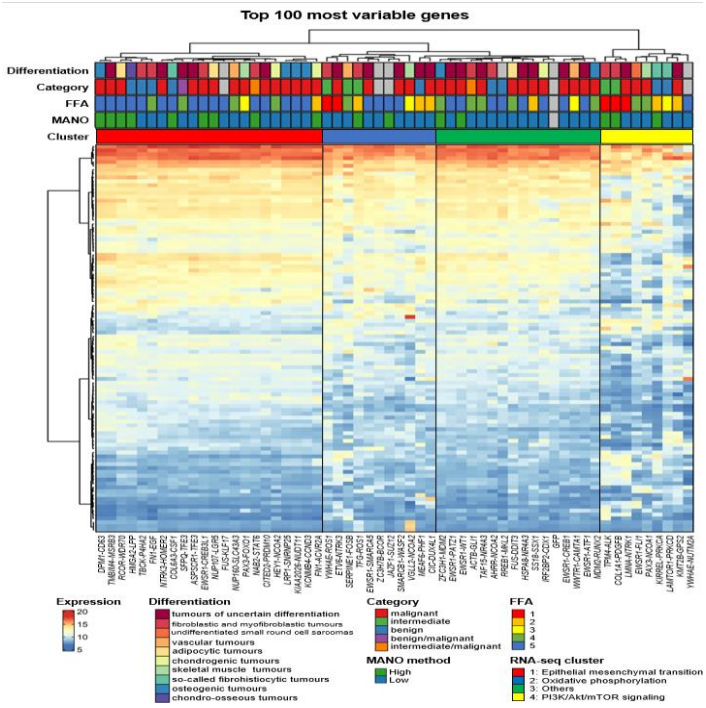
Methods: The transforming potential and drug sensitivities of these fusion genes were evaluated using a focus formation assay (FFA) and the mixed-all-nominated-in-one method (MANO method), respectively. The transcriptome was also examined using RNA-seq of 3T3 cells transduced with each fusion gene.

Results: Approximately half (28 of 59, 47%) of the fusion genes exhibited transformation in the FFA assay, which was classified into five types based on the resulting phenotype. The sensitivity to 12 drugs including multi-tyrosine kinase inhibitors (TKIs) was assessed using the MANO method and pazopanib was found to be more effective against cells expressing the COL1A1-PDGFB fusion gene compared with the others. The downstream MAPK/AKT pathway was suppressed at the protein level following pazopanib treatment. The fusion genes were classified into four subgroups by cluster analysis of the gene expression data and gene set enrichment analysis.

Conclusion: In summary, the oncogenicity and drug sensitivity of 59 fusion genes were simultaneously evaluated using a high-throughput strategy. Pazopanib was selected as a candidate drug for sarcomas harboring the COL1A1-PDGFB fusion gene. This assessment may be useful as a screening platform and provides a database to evaluate customized therapy for fusion gene-associated sarcomas.


ASPSCR1-TFE3
EWSR1-CREB3L1
EWSR1-FLI1
FUS-DDIT3
HEY1-NCOA2
HSPA8-NR4A3
NAB2-STAT6
PAX3-FOXO1
SFPQ-TFE3
SS18-SSX1
AHRR-NCOA2
TAF15-NR4A3
ACTB-GLI1
COL1A1-PDGFB
COL6A3-CSF1
ETV6-NTRK3
EWSR1-ATF1
EWSR1-CREB1
EWSR1-WT1
FN1-ACVR2A
KCNMB4-CCND3
KIAA2026-NUDT11
LMNA-NTRK1
LRP1-SNRNP25
MDM2-RUNX2
MEAF6-PHF1
NTRK3-HOMER2
NUP107-LGR5
NUP160-SLC43A3
RREB1-MKL2
TFG-ROS1
YWHAE-ROS1
ZFC3H1-MDM2
FUS-KLF17
CITED2-PRDM10
DPM1-CD63
TMBIM4-MSRB3
TPM4-ALK
WWTR1-CAMTA1
EWSR1-PATZ1
CIC-DUX4L1
RCOR-WDR70
HMGA2-LPP
SERPINE1-FOSB
FOSB-ZFP36
LAMTOR1-PRKCD
IRF2BP2-CDX1
KMT2B-GPS2
VGLL2-NCOA2
SMARCB1-WASF2
JAZF1-SUZ12
FUS-NFATC2
PAX3-NCOA1
KIRREL-PRKCA
ZC3H7B-BCOR
EWSR1-SMARCA5
TBCK-P4HA2
YWHAE-NUTM2A
FN1-EGF
GFP
ETV6-NTRK3
CIC-DUX4L1
WWTR1-CAMTA1
NUP160-SLC43A3
EWSR1-WT1
FFA
Type 1 2 3 4 5


(nM)
Viability
1.0
0.5
0
Doxorubicin
Crizotinib
Pazopanib
10
100
1000
10000
GFP
EWSR1-FLI1
HEY1-NCOA2
SFPQ-TFE3
SS18-SSX1
COL1A1-PDGFB
COL6A3-CSF1
ETV6-NTRK3
EWSR1-WT1
FN1-ACVR2A
KCNMB4-CCND3
KIAA2026-NUDT11
LRP1-SNRNP25
MDM2-RUNX2
NTRK3-HOMER2
NUP160-SLC43A3
TFG-ROS1
YWHAE-ROS1
ZFC3H1-MDM2
DPM1-CD63
TMBIM4-MSRB3
TPM4-ALK
CIC-DUX4L1
RCOR-WDR70
HMGA2-LPP
SERPINE1-FOSB
KMT2B-GPS2
VGLL2-NCOA2
JAZF1-SUZ12
PAX3-NCOA1
KIRREL-PRKCA
YWHAE-NUTM2A
EWSR1-SMARCA5
TBCK-P4HA2
FN1-EGF



(P 407) PRECISION AND PERSONALIZED TREATMENT FOR SOFT TISSUE SARCOMAS. RESULTS OF A 10 PATIENTS SERIES WITH MOLECULAR PROFILING USING NEXT-GENERATION MASSIVE SEQUENCING

PAULA MUÑOZ, Specialist; Elena Martínez, Phd; Alba Fernández, MD; Alejandro Paz, MD; Manuel Sureda, MD, PHD; Ramón G.Manzano, MD, PHD; Pedro Bretcha, MD, PHD; Antonio Brugarolas, MD, PHD, Hospital Quironsalud Torrevieja, Torrevieja, Comunidad Valenciana, Spain

Objective: Soft tissue sarcomas (STS) are a heterogeneous group of rare tumors with a diverse behavior and evolution. Surgery is the only curative treatment for this disease; however, multimodal, individualized treatment supported by molecular studies has improved the prognosis of these patients, especially in cases of advanced and metastatic disease. We present a 10 patient series illustrating a personalized and precision treatment guided by next-generation massive sequencing

Methods: Between January 2018 and January 2023, 10 patients with a diagnosis of soft tissue sarcoma were studied by massive DNA and RNA sequencing tumor genome with Ion Torrent platform (Oncomine Comprehensive Assay V3 panel) and tumor transcriptome (technique Ampliseq). All the cases were managed by a personalized multidisciplinary therapeutic approach: 2 cases with surgical planning through 3D reconstruction (Cella Medical Solution), 4 cases with intraoperative radiotherapy (IORT) administration, 1 case with isolated limb perfusion and 1 with electrochemotherapy treatment.

Results: The cases registered included: 3 retroperitoneal sarcomas, 3 extremity, 2 uterine, 1 splenic and 1 head and neck. 6 of them were primary, 1 metastatic, 1 recurrence, and 2 persistence due to initial surgery R2. We illustrate the precision treatment through 2

representative cases of a personalized treatment and therapeutic strategy guided by the molecular profile. Case 1: 73-year-old woman, January 2014 retroperitoneal leiomyosarcoma of the ureter - R0 en bloc resection with partial resection of the ureter and left hemicolectomy - Transcriptome-guided adjuvant treatment: Sorafenib and Riluzole due to GRM1 gene overexpression - Scapular recurrence with bone infiltration in Sept. 2019. Planning with 3D model, R0 wide resection, IORT application on bone margin - 2021 oligometastatic bilateral lung progression - Genome of metastasis: loss of function of SMARCB. Treatment with Tazemetostat in December 2022. Pulmonary disease in remission in April 2023. Overall survival: 108 months Case 2: A 49-year-old woman with pleomorphic sarcoma (UPS) of the left lower limb in October 2018. - Neoadjuvant chemotherapy and radiotherapy treatment, R0 wide surgery with adequate preservation of functionality and IORT - Early chest wall dermal/subcutaneous recurrence (resected) after initiation of immunotherapy – Nivolumab – due to a favorable profile for immunotherapy in tumor transcriptome - Genome of recurrence: BRAF V600E mutation. Treatment with Dabrafenib + Trametinib. - Complete remission of metastatic disease. April 2023 disease free. Overall survival: 64 months The molecular results of the 10 cases demonstrate the heterogeneity of these tumors and suggest new therapeutic possibilities. They will be presented in the final work.

Conclusion: STS are tumors with complex surgical and oncological management and a poor response to conventional chemotherapy protocols. Given its heterogeneity, precision oncology through molecular profiling with next-generation massive sequencing (panels for tumor genome and transcriptome) allows the development of personalized therapeutic strategies in patients with STS, and opens up the possibilities for targeted treatments for molecular alterations, immunotherapy and the development of new agents.

(P 408) CHANGES IN COMPUTED TOMOGRAPHY FEATURES INDUCED BY BRIGIMADLIN (BI 907828) IN PATIENTS WITH LIPOSARCOMA: INSIGHTS FROM PHASE I STUDIES

Lawrence H. Schwartz, MD[1]; Laurent Dercle, MD[2]; Binsheng Zhao, D.Sc.[2]; Rhea Chitalia, PhD[3]; Gisela M. Montgomery, MD, PhD[3]; Khanum Ridler, PhD[4]; Andrea Thiele, PhD[4]; Mehdi Lahmar, MD[5]; Patricia LoRusso, MD[6]; Noboru Yamamoto, MD[7]; Anthony W. W. Tolcher, MD[8]; Mrinal Gounder, MD[9], (1) Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY, (2) Radiology, New York-Presbyterian/Columbia University Medical Center, New York, NY, (3) Invicro, Needham, MA, (4) Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Baden-Wurttemberg, Germany, (5) Boehringer Ingelheim International GmbH, mainz, Rheinland-Pfalz, Germany, (6) Yale University School of Medicine, Yale Cancer Center, New Haven, CT, (7) Department of Experimental Therapeutics, National Cancer Center Hospital, Tokyo, Tokyo, Japan, (8) Clinical Research, NEXT Oncology, San Antonio, TX, (9) Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Traditional response assessment criteria such as RECIST are limited in evaluating treatment response in patients with liposarcoma receiving systemic therapy. The field of drug development for liposarcoma is witnessing a paradigm shift with the potential use of radiomics, a quantitative analysis of imaging features, for developing predictive and prognostic signatures. We aim to externally validate previously validated radiomic features identified in multicenter clinical trials to prove the concept of the potential of radiomics to provide valuable insights in liposarcoma drug development.

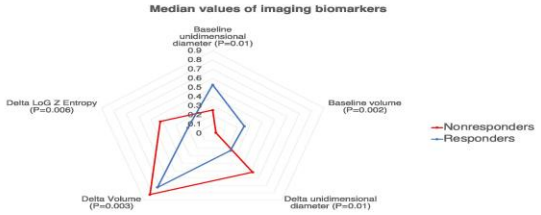
Methods: The cohort consisted of 22 patients, with 8 diagnosed with well-differentiated liposarcoma and 14 with dedifferentiated liposarcoma, treated with brigimadlin (BI 907828) in Phase 1 clinical trials NCT03449381 and NCT03964233. These patients had a total of 40 measurable tumor lesions based on CT scans. Radiologists performed semi-automatic segmentation of the tumor lesions at all available time points and extracted tumor features using both Columbia Image Feature Extractor (CIFE) and pyRadiomics software. Ten pre-identified prognostic and/or predictive radiomic features extracted at baseline and Week 6 were used to compute delta features reflecting changes between these time points, with the objective of differentiating responders (patients with a partial or complete response based on RECIST 1.1 criteria) from non-responders through per-patient analysis (n=22) and per-lesion analysis (n=40). As an ancillary study, independent radiologists assessed qualitative features such as solidity, necrosis, shape, calcification, vascularization, and septations.

Results: A majority of patients (16 out of 22) had tumors located in the retroperitoneum. The dose regimen for brigimadlin in this study varied among the enrolled patients. In the per-patient analysis, at baseline, we observed that responders (P-value of patient-based/lesion-based analyses) had higher tumor volume (P=0.002/< 0.0001) and a distinct radiomics phenotype (Figure 1). The analysis of early changes in tumor phenotype demonstrated that responders had a decrease in both tumor volume (P=0.003/0.001) and in tumor heterogeneity (P=0.006/0.003). In line with the quantitative radiomics analysis, treatment response was more likely to be observed in qualitatively predominantly fatty lesions and in those with fewer and thinner septae.

Conclusion: Based on a small patient cohort, the radiomic analysis hints at the potential of the selected features to predict treatment response at both patient and lesion levels, with the most significant feature being the reduction in tumor volume and heterogeneity on CT scans at Week 6. The validation of these radiomic features, previously identified in multicenter trials, indicates their potential in identifying patients who will benefit from treatment; this was further supported by the qualitative assessment performed by radiologists.

Figure. Comparison of imaging biomarkers in responders and nonresponders

The figure displays the median values for five imaging features: Uni (mm), Volume (mm³), Delta_Uni (mm), Delta_Volume (mm³), and Delta_>LoG_Z_Entrop>Para_1. Scaled median values were computed using the formula zi = (xi – min(x)) / (max(x) – min(x)), where xi represents the original value, min(x) is the minimum value of the feature, and max(x) is the maximum value of the feature. Scaled values are displayed on the graph to compare responders and nonresponders. Responders had higher baseline tumor volume, greater tumor volume shrinkage, and distinct change in tumor heterogeneity (Delta Log Z Entropy).



	Uni (mm)	Volume (mm3)	Delta Uni (mm)	Delta Volume (mm3)	Delta LoG Z Entropy
Median in nonresponders	70	95452	1	44	0.02
Median in responders	123	923618	-25	-234161	-0.19
Minimal value	22	4188	-45	-1993400	-0.37
Maximal value	213	3602050	43	430880	0.55
Scaled median in nonresponders	0.25	0.03	0.52	0.82	0.42
Scaled median in responders	0.53	0.26	0.23	0.73	0.20

(P 409) IMMUNOHISTOCHEMICAL STAINING FOR MYOGENIN, AP2 BETA AND HMGA2 PREDICT FOXO1 FUSION STATUS IN RHABDOMYOSARCOMA: A REPORT FROM THE SOFT TISSUE COMMITTEE OF THE CHILDREN'S ONCOLOGY GROUP

Sonja Chen, MD[1]; Wei Xue, PhD[2]; Abha Gupta, MD MSc[3]; Roshni R. Dasgupta, MD MPH[4]; Suzanne Wolden, MD[5]; Rajkumar Venkatramani, MD[6]; Archana Shenoy, MD[1]; Michael Arnold, MD PhD[7]; Lisa a. Teot, MD[8]; Erin Rudzinski, MD[9], (1) Nationwide Children's Hospital/The Ohio State University College of Medicine, Columbus, OH, (2) University of Florida, Gainsville, FL, (3) Princess Margaret Cancer Center, Toronto, ON, Canada, (4) Cincinnati Children's Hospital Medical Center, Cincinnati, OH, (5) Memorial Sloan Kettering Cancer Center, New York, NY, (6) Baylor College of Medicine / Texas Children's Hospital, Houston, TX, (7) Children's Hospital of Colorado/University of Colorado, Aurora, CO, (8) Boston Children's Hospital and Harvard Medical School, Boston, MA, (9) Seattle Children's Hospital/University of Washington, Seattle, WA

Objective: Presence of a FOXO1 fusion is a key prognostic factor in rhabdomyosarcoma (RMS). Based on prior gene expression studies, surrogate immunohistochemical (IHC) markers have been proposed that discriminate between FOXO1 fusion positive and FOXO1 fusion negative RMS with high specificity. IHC staining was incorporated into central pathology review for ARST1431 (Children’s Oncology Group intermediate risk RMS trial supported by Pfizer) to evaluate the performance of this IHC panel in a prospective cohort of patients with known fusion status.

Methods: Central pathology review for patients enrolled on ARST1431 included review of H&E morphology as well as staining characteristics for myogenin, AP2beta and HMGA2. Based on prior published algorithms, the pattern of IHC staining was used to predict the presence or absence of a FOXO1 gene fusion. Specifically, presence of a FOXO1 fusion was predicted if myogenin staining was present in greater than 50% of tumor cells and AP2beta staining was subjectively stronger than HMGA2 staining. IHC fusion prediction was then compared to the results of institutional fusion testing.

Results: Pathology review was completed for 306 patients, of whom 58%, 29% and 13% had embryonal, alveolar or other morphology, including spindle cell, mixed and NOS, respectively. 98% (299/306 cases) had sufficient tissue for central IHC testing, of which 292 had assessment of fusion status. IHC testing was not interpretable in 1 case in which the tissue was necrotic. Institutional assessment of fusion status included fluorescence in situ hybridization in 85% (248/291), RT-PCR in 7% and a variety of NGS, karyotyping or Nanostring in the remainder. Using the previously described algorithm, the sensitivity of IHC to predict fusion status was 98.7% and specificity was 97.6%. True positive cases included 71 ARMS, 5 RMS, mixed and 2 RMS, NOS. True negative cases included 170 ERMS, 7 ARMS and 29 others including 22 SSRMS and 7 RMS, NOS. False negative cases included 1 RMS, NOS. False positive cases comprised 5 morphologic ARMS; additional molecular profiling is underway in all cases with available material.

Conclusion: Surrogate marker IHC has high sensitivity and specificity to predict FOXO1 fusion status in RMS and may be useful in small biopsies with scant or poor quality material. Additional analysis of staining patterns in specific biologic subtypes (eg. MYOD1 positive RMS) and analysis of fusion variants is ongoing.

(P 410) PROTEOMICS ANALYSIS IDENTIFIES A SIGNATURE THAT IMPROVES PROGNOSTIC RISK STRATIFICATION OF THE SARCULATOR NOMOGRAM IN PATIENTS WITH SOFT TISSUE SARCOMAS OF THE EXTREMITIES AND TRUNK WALL

Madhumeeta Chadha, MSc[1]; Sara Iadecola, PhD[2]; Jessica Burns, PhD[1]; Amani Arthur, MBChB BSc(Hons) MRCPCH[1]; Dario Callegaro, MD[3]; Khin Thway, MD[4]; Alessandro Gronchi, MD, FSSO[5]; Robin L. Jones, BSc, MB BS, MRCP, MD[6]; Rosalba Miceli, MD, PhD[2]; Sandro Pasquali, MD, PhD[3]; Paul Huang, PhD[1], (1) The Institute of Cancer Research, London, England, United Kingdom, (2) Unit of Biostatistics for Clinical Research Department of Epidemiology and Data Science, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (3) Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (4) The Royal Marsden NHS Foundation Trust, London, England, United Kingdom, (5) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (6) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom

Objective: High-risk patients with soft tissue sarcomas of the extremities and trunk wall (eSTS), as defined by the Sarculator nomogram, are more likely to benefit from (neo)adjuvant anthracycline therapy compared to low/intermediate-risk patients. Nomogram predicted risk groups may have distinct biology that may explain their differential outcome after treatment. Here we used high resolution mass spectrometry (MS) to define the protein networks that are characteristic of nomogram-stratified risk groups and evaluate a protein signature based on DNA replication as a complementary prognostic tool to the Sarculator nomogram.

Methods: Proteomic data of primary surgical specimens from 123 eSTS patients across 8 histological types was acquired using MS. A Cox model for overall survival (OS) including the Sarculator nomogram was fitted to individual data to generate predicted probability of 5-year Overall Survival (pr-OS), which were used to define 4 quartile-based risk categories. This allowed us to recalibrate the baseline survival to the present case series. Proteins that were significantly enriched in different risk groups were identified by Wilcoxon-Mann-Witney test with multiple testing correction. Biological pathways that were upregulated in high-risk versus low-risk patients were defined using gene set enrichment analysis (GSEA). The expression pattern of the DNA replication signature - Sarcoma Proteomic Module 6 (SPM6) was evaluated for association with tumour grade (Wilcoxon test), histological type and nomogram risk groups (Kruskal-Wallis test). SPM6 was added as a covariate together with the Sarculator nomogram in a multivariable Cox model. Discriminative ability of the Sarculator only or Sarculator + SPM6 Cox models was evaluated by Harrell C index while Index of Prediction Accuracy (IPA) was used to evaluate discrimination and calibration. Agreement between predicted and observed OS was assessed using calibration plots.

Results: Quartiles were applied as cut-offs to pr-OS for identifying four categories corresponding to high risk (pr-OS≤39%), intermediate risk (39%< pr-OS≤52%), low risk (52%< pr-OS≤69%) and very low risk (pr-OS>69%) patients. Supervised clustering of the proteomic dataset of 3384 proteins of the 4 nomogram predicted risk groups is shown in Figure 1A. Analysis of proteins enriched in high risk versus very low risk patients identified components of the minichromosome maintenance (MCM) complex to be significantly upregulated. We previously defined 14 sarcoma proteomic modules which comprise co-expressed proteins across a range of biological functions. In particular, the SPM6 protein signature comprises 41 DNA replication proteins that includes the MCM complex (Figure 1B). SPM6 median protein expression levels are significantly associated with tumour grade (p = 1.6e-06), histological type

(p = 1.4e-05) and nomogram predicted risk groups (p = 2.6e-06). Cox model analysis showed that SPM6 slightly improved the discriminative ability of the Sarculator nomogram (Harrell C index=0.69 for Sarculator nomogram alone vs 0.698 for Sarculator nomogram + SPM6). As regards to calibration, while the nomogram only predictions were quite accurate, despite the baseline survival recalibration there was some discrepancy between predictions and true outcomes particularly in the intermediate and very low risk groups (Figure 1C left) and addition of SPM6 as a covariate substantially contributed to a better calibration (IPA =0.109 vs 0.165; Figure 1C right).

Conclusion: We have performed the first molecular analysis of the biological pathways characteristic of nomogram predicted risk groups in eSTS. We further show that the SPM6 DNA replication signature improves prognostic risk stratification of the nomogram. While hypothesis generating, this study highlights the future potential of integrating protein signatures with Sarculator for the development of next-generation nomograms.

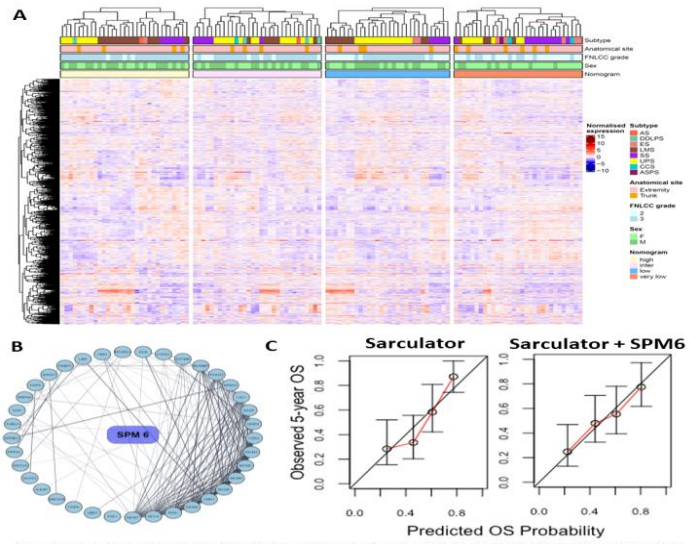


Figure 1 (A) Annotated heatmap of supervised analysis of 3,384 proteins across the four Sarculator predicted risk groups. Within each risk group, cases were subjected to unsupervised clustering (Pearson's distance). AS = angiosarcoma, ASPS = alveolar soft part sarcoma, CCS = clear cell sarcoma, DDLPS = dedifferentiated liposarcoma, ES = epithelioid sarcoma, LMS = leiomyosarcoma, SS = synovial sarcoma, UPS = undifferentiated pleomorphic sarcoma. **(B)** Protein-Protein Interaction (PPI) network of SPM 6 comprising 41 nodes and 162 edges that includes the MCM complex. Nodes indicate proteins and edges indicate StringDB database score between proteins, where a thicker line indicates a higher score. **(C)** Calibration plots for Sarculator only (left) and Sarculator + SPM6 Cox models of overall survival (OS) 5-year predictions. The predicted probabilities were stratified into four risk groups. For each risk group, the average predicted probability (x-axis) was plotted against the Kaplan-Meier estimate (y-axis). 95% Confidence Intervals of the Kaplan-Meier estimates are indicated with vertical lines. The diagonal line is the reference line, indicating the ideal nomogram.

(P 411) A SPATIAL TRANSCRIPTOMIC "GOOGLE MAP" OF HUMAN OSTEOSARCOMA REVEALS COMMON CELL CLUSTERS AT THE PRIMARY SITE AND PULMONARY METASTASES.

Luigi Mancinelli, PhD[1]; Karen E. Schoedel, MD[2]; Kurt R. Weiss, MD[1]; Giuseppe Intini, D.D.S., PhD[1], (1) University of Pittsburgh, Pittsburgh, PA, (2) University of Pittsburgh Medical Center, Pittsburgh, PA

Objective: Osteosarcoma (OS) is the most common primary malignant bone tumor with poor prognosis. As the survival rate for patients with metastases still remains at only 20%, new research approaches are needed to better understand the mechanisms of their progression.

Through spatial transcriptomic analysis of two pairs of matching primary and lung metastases (LM) of osteosarcoma obtained from two patients, we aimed to describe the identity, localization, transcriptomic profiles, and developmental interplay of tumor and non-tumor cells and of cells residing at the tumor-non tumor interface.

Methods: Samples were obtained from a 19yo female (Patient 1-P1) and a 15yo male (Patient 2-P2) diagnosed with high-grade conventional osteosarcoma. Both patients received neoadjuvant chemotherapy: 20% tumor necrosis was achieved in P1, and 92% tumor necrosis was achieved in P2. Upon collection, samples were fixed for 28h in PFA 4%, embedded in paraffin, and sectioned at 5µm. H&E staining was performed to evaluate tissue integrity and to select target areas containing both tumoral and non-tumoral tissue. RNA degradation of the tissue samples was assessed by means of DV200 (DV200 ≥ 50%). Selected sections were processed with 10x Genomics Visium Spatial Gene Expression for FFPE samples® (10X Genomics, Inc. USA) according to the provided protocol.

Results: The unbiased cluster analysis of all samples identified 12 major clusters, including clusters of osteogenic, immune, epithelial, and endothelial cells, and adipocytes. Boundaries of the clusters overlapped with the edges of the histological regions identified by the pathologist (Fig. 1 A, B). Three clusters of malignant osteosarcoma cells (MOCs) presenting an osteogenic-like phenotype were detected in the tumoral areas (Fig. 1B, C): MOCs-1 was found in all primary sites and pulmonary metastases; MOCs-2 was observed in the primary OS of P1 and in LM of both patients; and MOCs-3 was found only in the primary OS of P2 (Fig. 1B, C). Pathway analysis of MOCs-1 revealed a significant decrease in apoptosis and cell death signaling along with a pronounced increase of cell invasion and migration signaling. Similar observations were made for MOCs-2. In addition, a comparison between MOCs-1 of the primary OS cells vs MOCs-1 of the LM revealed that MOCs-1 of the metastases express higher levels of genes typically present in the lung (SFTPB, SFTPC) (Fig. 2A) which are absent in primary OS. Similar results were obtained by analyzing MOCs-2.

Immune cell clusters were found mainly in the LM localized at the tumor-lung interface in both patients, while primary tumors did not harbor significant immune infiltrates. Low expression of CD8, cytotoxic T-lymphocyte antigen 4 (CTLA4), and programmed cell death 1 (PD-1) was detected in all samples, indicating a limited T-cell infiltration within the tissues. Also, PD-L1 was minimally expressed (Fig. 2B). However, by looking at the gene expression profile of MOCs-1 and MOCs-2, we found remarkable expression of genes involved in carcinogenesis, cell transformation, and cell adhesion (YAP1, GREM1, and CDH11).

Conclusion: Our data show that the MOCs-1 and MOCs-2 of LM display expression of lung-specific genes while maintaining a high level of similarity with MOCs-1 and MOCs-2 of primary OS, suggesting that these genes may be responsible for the metastases' onset and development within the lungs. The lack of significant T-cell infiltrates in all samples may explain the limited efficacy observed in OS clinical trials targeting the PD-1/PD-L1 pathway. YAP1, GREM1, and CDH11 being potentially targetable, may identify novel therapeutic avenues.

This study, for the first time, presents a spatial transcriptomic analysis of primary and metastatic lesions of human OS, in the form of a consultable "Google Map", offering the opportunity to retrieve useful information about the localization, characteristics, and developmental dynamics of the cells of human OS.

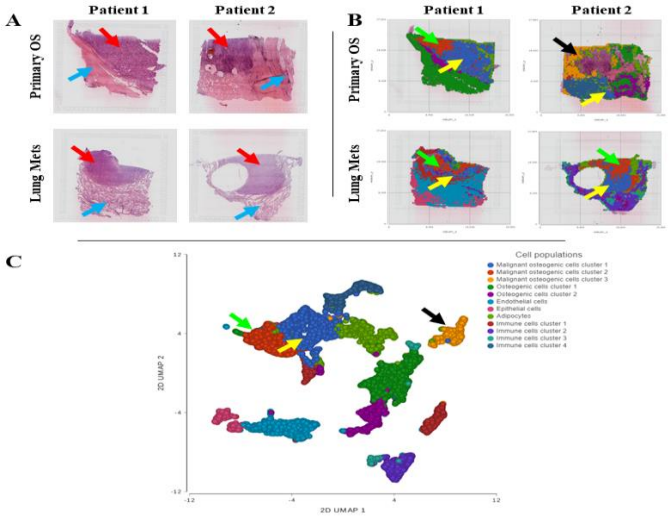


Fig 1. Spatial transcriptomic analysis of two pairs of matching primary osteosarcoma (OS) and lung metastases isolated from two patients. (A) H&E staining showing histology and localization of tumoral tissue (red arrows) and non-tumoral tissue (blue arrows) in both primary OS and lung metastases. **(B)** Unbiased cluster analysis showing cell populations and their localizations in both primary OS and lung metastases. The analysis revealed three clusters of malignant osteogenic cells (MOCs) all found to reside in the tumoral tissue: MOCs-1 (yellow arrows) was detected in all primary and metastases lesions; MOCs-2 (green arrows) was found in primary OS of patient 1 and in lung metastases of both patients; MOCs-3 (black arrow) was observed only in the primary OS of patient 2. **(C)** Uniform manifold Approximation and Projection (UMAP) showing cell populations of both primary OS and lung metastases. Yellow, green and black arrows are pointing to MOCs-1, MOCs-2 and MOCs-3, respectively.

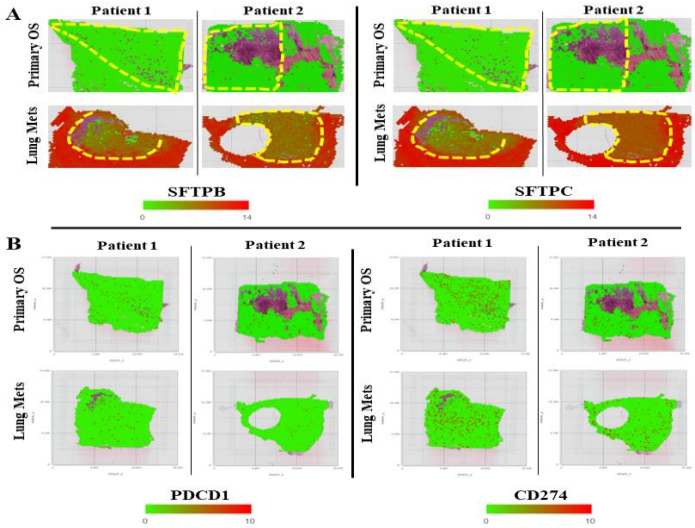


Fig 2. Gene expression analysis in matching primary osteosarcoma (OS) and lung metastases isolated from two patients. (A) Scatter plot showing gene expression and localization of (left) surfactant protein B (SFTPB) and (right) surfactant protein C (SFTPC) in malignant osteosarcoma cells (MOCs); yellow dashes underline the tumoral tissue where MOCs reside in both primary OS and lung metastases; bottom bars indicates gene expression level from low (green) to high (red). **(B)** Scatter plot showing gene expression and localization of (left) programmed cell death 1 (PDCD1) and (right) programmed cell death ligand 1 (CD274) in both primary OS and lung metastases; bottom bars indicates gene expression level from low (green) to high (red).

(P 412) LEIOMYOSARCOMA POLYANEUPLOID CANCER CELLS DEVELOP IN RESPONSE TO CHEMOTHERAPY AND CONTRIBUTE TO CHEMORESISTANCE

Lisa M. Abegglen, PhD[1]; Ryan Kennington, BS[2]; Gareth Mitchell, BMus, MBA[3]; Niraja Bhachech, MS[3]; C. Brad Nelson, BS[3]; Emily Payne, BS[4]; Miranda Sharp, BS[3]; Anthony Iovino, BS[3]; Aarushi Rohaj, BS[3]; Sarah R. Amend, PhD[5]; Kenneth J. Pienta, MD[5]; Jonathan A. Fletcher, MD[6]; Matt van de Rijn, M.D.[7]; Ting Liu, MD[3]; Joshua D. Schiffman, MD[3], (1) Huntsman Cancer Institute/University of Utah, North Salt Lake, UT, (2) Huntsman Cancer Institute/University of Utah, 84112, UT, (3) Huntsman Cancer Institute/University of Utah, Salt Lake City, UT, (4) Huntsman Cancer Institute/University of Utah, (5) Johns Hopkins School of Medicine, Baltimore, MD, (6) HarvardMedical School/Brigham and Women's Hospital, Boston, MA, (7) Stanford University, Stanford, CA

Objective: Polyaneuploid cancer cells (PACCs) are large, treatment resistant cancer cells identified in different cancer types, sometimes called “giant cells.” PACCs have stem cell-like phenotypes and appear to play a role in treatment resistance that leads to poor patient outcomes. The PACC phenotype is a transient state that cancer cells can adopt to protect themselves from stress. PACCs give rise to non-PACC progeny that maintain resistance to chemotherapy. While PACCs are documented in many cancer types, they have not yet been documented in sarcoma. We identified PACCs for the first time in cell culture from leiomyosarcoma (LMS) patient tumors. The current study characterizes LMS PACCs in vitro, in vivo, and in patient samples. This effort may identify precision-based approaches to therapeutically target LMS PACCs.

Methods: LMS PACCs in vitro: LMS01, LMS03, LMS04, and LMS05 cells were treated with doxorubicin, docetaxel, or gemcitabine for 7 days followed by 7 days without chemotherapy to enrich for PACCs. PACC progeny were then generated by culturing the enriched LMS PACCs for 1-2 weeks without chemotherapy. Cells were imaged before treatment (parent cells) and after 7 days of chemotherapy treatment followed by 7 days without drug (PACCs). Cell size, DNA content, and in vitro response to chemotherapy were measured and compared between parent, PACC, and PACC progeny.
LMS PACCs in vivo: In vivo PACC formation was assessed by xenograft. LMS tumors were grown in mice, followed by treatment with doxorubicin. Tumors were harvested 4-5 days post-treatment and imaged for PACC formation by staining with DAPI (DNA) and CD47 (tumor cell marker).
LMS PACCs in human patients: LMS human patient microarrays were stained with H&E and analyzed by a pathologist to determine the percent of samples with giant cells and the percent with abnormal nuclei.
LMS PACC response to p53 expression: LMS PACC cells were transfected to express elephant p53 or mCherry with or without doxorubicin and apoptosis was quantified.

Results: LMS cells treated in vitro and in vivo with chemotherapy (doxorubicin, gemcitabine, or docetaxel) led to the development of large cells with atypical nuclei as the predominant cellular phenotype (Figures 1 and 4). In vitro, these LMS PACCs were more resistant to chemotherapy (Figure 2) and repopulated the culture with non-PACC LMS progeny cells potentially through a budding process called neosis. These LMS PACC progeny cells maintained a significantly decreased ($p < 0.0001$) response to chemotherapy compared to parent cells (Figure 3). PACCs were identified in clinical samples from patients with LMS (Figure 4). 29% of LMS samples contained abnormally large tumor cells and 53% contained abnormal nuclei. LMS PACCs transfected with functional TP53 undergo rapid apoptosis upon gene expression. Additionally,

combining functional TP53 expression with low doses of doxorubicin increases the apoptotic response of LMS cells, potentially by targeting PACCs.

Conclusion: We offer the first description and in-depth characterization of LMS PACCs. These large, non-dividing cells are chemoresistant and may contribute to the lack of clinical response often seen in LMS. Current research efforts are focused on correlating LMS PACCs in clinical samples with initial response to therapy, as well as long-term patient outcomes. Identifying therapeutic approaches to target and kill LMS PACCs may improve patient response to chemotherapy and clinical outcome. Given the very high rate of TP53 involvement in LMS, TP53 loss may contribute to the ability of LMS PACCs to form abnormal, multiple nuclei. Ongoing investigations may support the development of effective TP53 based therapeutics to target PACCs and to improve outcomes for LMS patients.

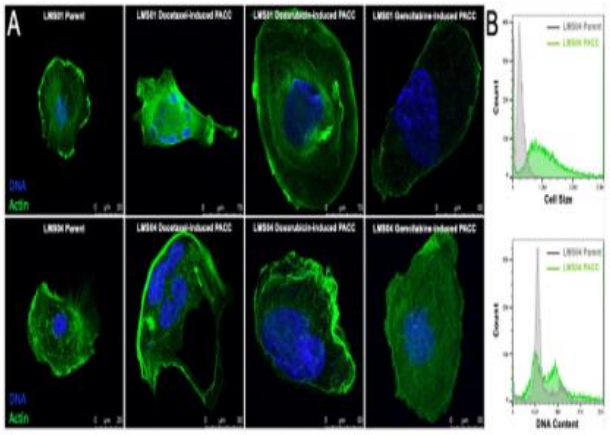


Figure 1: LMS cells increase in size and DNA content as measured by imaging (A) and flow cytometry (B).

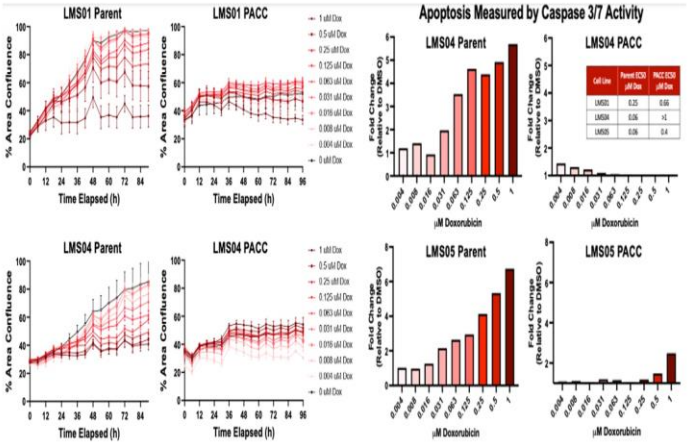


Figure 2: LMS PACCs do not proliferate or undergo apoptosis in response to doxorubicin.

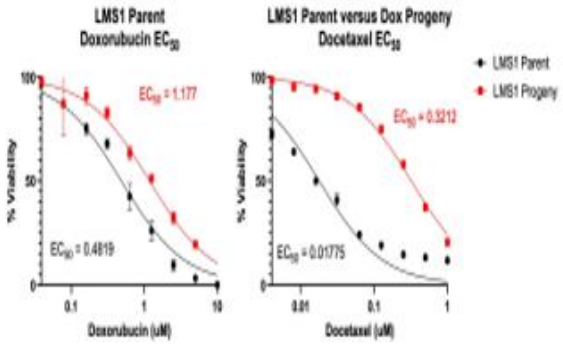


Figure 3: LMS PACC progeny are more resistant to doxorubicin and docetaxel than LMS parent cells.

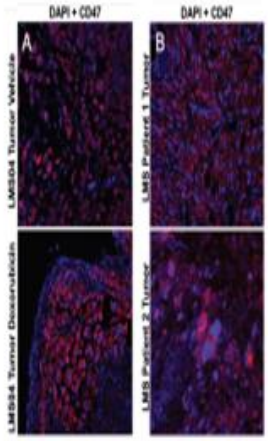


Figure 4: Large LMS cells with abnormal nuclei *in vivo*. LMS tumors grown in mice and treated with doxorubicin (A) and LMS patient samples without (top) and with (bottom) large cells with abnormal nuclei (B).

(P 413) LINKING REAL-WORLD CLINICAL AND GENOMIC DATA IN RHABDOMYOSARCOMA: LEVERAGING AACR PROJECT GENIE AS A PATH TOWARD PRECISION ONCOLOGY IN RARE CANCERS

Amanda Marinoff, MD; Kelly Blum, MS; Mi-Ok Kim, PhD; Michelle Turski, PhD; Alejandro Sweet-Cordero, MD; Amit Sabnis, MD, UCSF, San Francisco, CA

Objective: Rhabdomyosarcomas (RMS) are common soft tissue sarcomas in childhood, adolescence, and less frequently in adults. The WHO recognizes four histopathologic subtypes of RMS, and landmark studies have begun to identify genomic features associated with each. Still, the diverse clinical features and outcomes within this large patient population suggests that advancements in prognostic precision and treatment strategies may be improved by a deeper understanding of the genomic drivers and modifiers of disease and their associations

with clinical phenotypes. Aside from FOXO1 fusion status in pediatric RMS, there are few genomic prognostic features available for risk stratification, and the clinico-genomic landscape of adult RMS, a rare cancer with poor outcomes, has not been well characterized. Utilizing Project GENIE, an international real-world clinico-genomic data-sharing consortium, we report on recurrent genomic features of RMS across the age spectrum and assess their associations with clinical phenotypes.

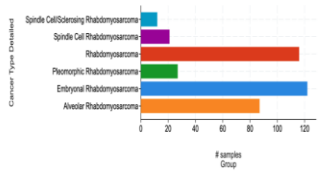
Methods: We analyzed targeted next-generation sequencing data of 388 tumors from 351 patients with RMS in the AACR Project GENIE registry, which aggregates, harmonizes, and links clinical-grade next-generation sequencing data with clinical data from 19 cancer institutions, each with a unique targeted panel (45-531 genes). The majority of data are from patients treated at 3 cancer centers: Memorial Sloan Kettering, Dana-Farber Cancer Institute, and University of California San Francisco. The registry currently contains 167,000 samples and is projected to reach >250,000 by 2025. We assessed the publicly available clinical and genomic features of this real-world cohort. To evaluate associations between genomic features and more detailed clinical phenotypes, we performed deeper clinical annotation on a subset of samples using PRISSMM (Pathology, Radiology/Imaging, Signs/Symptoms, Medical oncologist assessment, and tumor Markers), a novel curation framework for electronic health record abstraction at scale. Logistic regression was used to assess clinical and genomic associations, and p-values were adjusted for the false discovery rate.

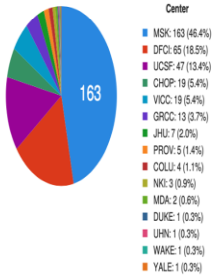
Results: Fifty-seven percent of patients were < 18 years, and 43% were ≥18 years at the time of sequencing (range < 18 to 86 years). Fifty-eight percent of patients were deceased at the time of last follow-up. Sixty-eight percent of samples were primary tumors and 32% were metastatic samples. The median number of mutations per sample was 3 (range 1 to 39). Fourteen percent of patients had FOXO1 fusion positive RMS, 65% of whom were < 18 years at the time of sequencing. Amongst patients > 18 years, the mean and median age of FOXO1 fusion positive patients was 32 and 24 years, respectively (range 20-78 years), demonstrating a high prevalence even in adult RMS patients. The most frequently altered genes across the cohort were TP53, FOXO1, NF1, NRAS, BCOR, and CDKN2A. In fusion negative tumors, RAS pathway alterations were detected in >50% of cases, and cell cycle regulation pathways were altered in the remaining cases. TP53 and MDM2 alterations were found exclusively in fusion negative tumors. TP53 alterations (19%), detected at a higher frequency than previously reported, were also significantly enriched in pleomorphic RMS ($q < 0.001$).

Conclusion: This is the first study to genomically characterize RMS across the age continuum and to utilize publicly available real-world patient data to assess clinico-genomic associations in a large cohort of pediatric cancer patients. We demonstrate the feasibility of using such an approach to define genomic features in heterogeneous, rare cancers and highlight the value of real-world data collaboration in advancing precision oncology.

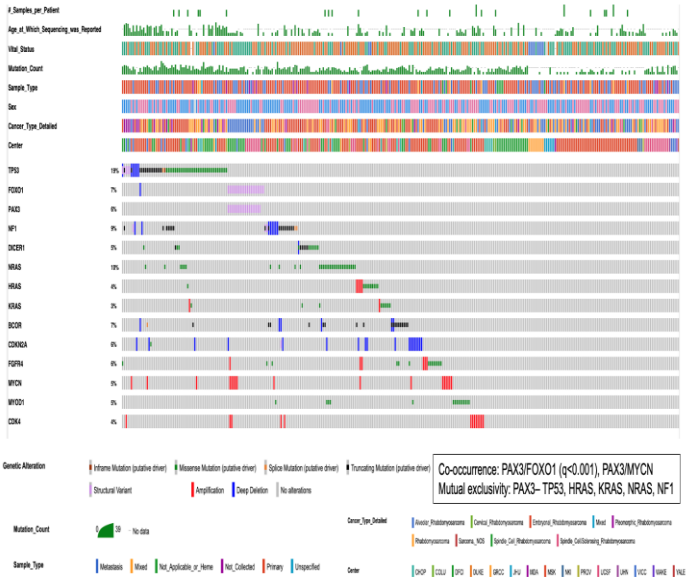
Characteristics of 388 samples from 351 patients with rhabdomyosarcoma (RMS)

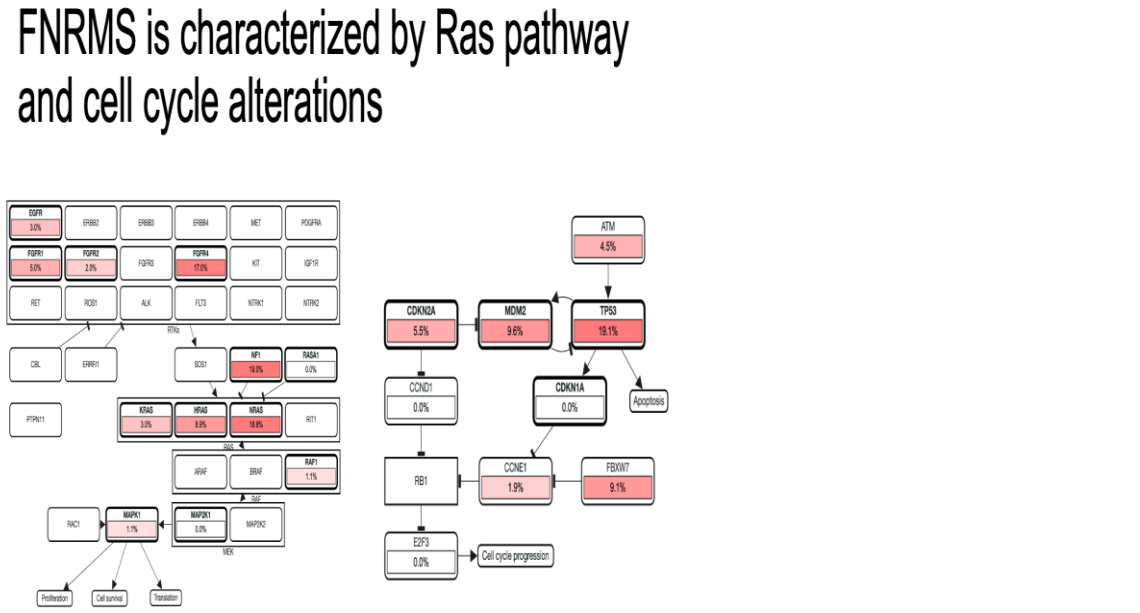
Characteristic	No. (%)
Sex	
Female	175 (45.1%)
Male	212 (54.6%)
Vital Status	
Deceased	217 (58.3%)
Alive	155 (41.7%)
Unknown	16 (4.1%)
Age at Sequencing	
Age≥18	167 (43.4%)
Age<18	218 (56.6%)
Unknown	3 (0.8%)
Primary ethnicity	
Non-Spanish/non-Hispanic	270 (69.6%)
Spanish/Hispanic	57 (14.7%)
Unknown	61 (15.7%)
Primary race	
Asian	16 (4.1%)
Black	35 (9.0%)
Native American	1 (0.3%)
Other	24 (6.2%)
Pacific Islander	1 (0.3%)
White	239 (61.6%)
Number of Samples Per Patient	
Mean (SD)	1.27 (0.602)
Median [Min, Max]	1.00 [1.00, 6.00]
Unknown	77 (19.8%)
Sample Type	
Primary	246 (69.1%)
Metastasis	110 (30.9%)
Unknown	32 (8.2%)





Recurrent genomic alterations in 351 RMS samples in GENIE characterized by targeted NGS





(P 414) GENOMIC ANALYSIS OF EWING SARCOMA AND HERNIA IDENTIFIES A POSITIVE GENETIC CORRELATION AND SUGGESTS BOTH ARE LINKED TO AN HMGA2 VARIANT

Logan G. Spector, PhD[1]; Tianzhong Yang, Ph.D.[1]; Lauren J. Mills, PhD[1]; Aubrey K. Hubbard, Ph.D.[2]; Rui Cao, M.S.[3]; Andrew Raduski, Ph.D.[1]; Mitchell J. Machiela, PhD[4], (1) University of Minnesota, Minneapolis, MN, (2) National Cancer Institute, Bethesda, MD, (3) University of Minnesota, Falcon Heights, MN, (4) National Cancer Institute, Rockville, MD

Objective: The literature on the causes of Ewing sarcoma (EWS) is exceedingly limited, but a few clues have emerged with surprising consistency. In particular, a 2005 meta-analysis by Valery et al (PMID: 15992697) examined five case-control studies of EWS and found significant associations between this rare cancer and umbilical [odds ratio (OR) = 3.3; 95% confidence interval (CI): 1.3-8.0] and any hernia [OR = 3.2; 95% CI: 1.3-8.0]. Hernias are heterogeneous defects but all are characterized by the intrusion of a tissue or organ into an adjacent, inappropriate space; many are also considered developmental defects. While associations with hernias could expand understanding of the etiology of EWS, few epidemiologic studies of EWS have further examined the association. Recently, the genetic epidemiology of EWS has begun to be characterized, most recently in a genome-wide association study (GWAS) by Machiela et al (PMID: 30093639) who identified six germline susceptibility loci with genome-wide significance and strong per-allele ORs. The genetic epidemiology of hernias has also come into focus, with Fadista et al (PMID: 35680855) describing 81 independent loci for five types of hernia, many of which overlap. With these two datasets we aimed to conduct a comprehensive analysis of the genetic relationship between different types of hernia and EWS.

Methods: We used summary statistics from a GWAS of 733 EWS cases and 1346 controls

conducted by Machiela et al and the summary statistics derived from the GWAS study of 28707, 31193, 6402, 1049, and 4644 inguinal, diaphragmatic, umbilical, femoral and ventral hernia cases, respectively, and 65,492 controls in the UK Biobank conducted by Fadista et al. All datasets focused on Europeans. We analyzed SNPs with minor allele frequency > 1% in all datasets. We evaluated the globalized and localized relationship between EWS and each hernia type, using Bonferonni adjustment for multiple testing. On the global level, we estimated the pairwise genetic correlation by linkage disequilibrium score (LDSC) regression and the casual relationship by Mendelian Randomization analysis (MR), In the MR analysis, we examined the model assumptions by checking for directional pleiotropy, strength of the instrumental variables (IV), heterogeneity, and filtered SNPs which failed Steiger's directional test. We used a random-effect, inverse-variance weighted (IVW) method as the primary analysis whereas MR Egger, weighted mean, simple mode, and weighted mode MR were performed as sensitivity analyses.

On the regional level, we used the Multi-Trait Analysis of GWAS (MTAG) algorithm that leverages the genetic correlation among different hernias to improve the GWAS estimates for each hernia type except femoral hernia, based on which we constructed the COLOC algorithm on the regions 10,000 base pair upstream and downstream from the six genome-wide significant EWS variants (rs11366169, rs7742053, rs10822056, rs2412476, rs6047482, rs6106336) and the genome-wide significant lead SNPs for hernias identified from the MTAG estimates. The resulting test for colocalization establishes whether the same variant is associated with both phenotypes. We further estimated the local genetic covariance for the LD blocks around these regions using the SUPERGNOVA algorithm.

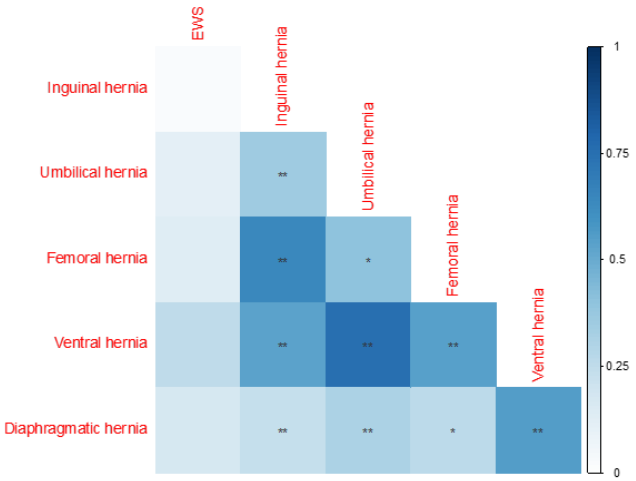
Lastly, we examined colocalized SNPs for evidence of being tumor expression quantitative trait loci (eQTL) using tumor RNA-seq and germline array data generated by Crompton et al (PMID: 25186949) and downloaded from dbGap (phs000804).

Results: The five hernia types were significantly correlated with each other on a global level, except for a few marginally significant pairs (Figure 1). None of the hernia had a statistically significant genetic correlation with EWS. However, all estimates suggested a positive global relationship, among which ventral hernia had the strongest global correlation with EWS (genetic correlation = 0.26) and inguinal hernia had the weakest association (genetic correlation = 0.02).

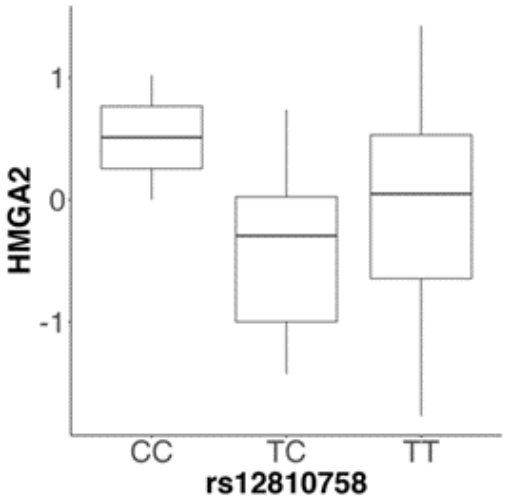
In the MR analyses, the odds of EWS was 2.26 higher among those with diaphragmatic hernia compared to those without and was statistically significant [95% confidence interval (CI) = 1.30,3.95, p-value = 0.004]. Additionally, we observed a marginally significant causal relationship between inguinal hernia and EWS with an OR = 1.27, 95% CI = (1.01, 1.59), and p-value = 0.041. There was no indication of violation of model assumptions and no concern for having weak IV. The MR analysis on single SNPs suggests that the significance was mainly driven by two SNPs, one of them was rs12810758.

Among six SNPs associated with EWS and 120 SNPs associated with any of the hernia in MTAG, we identified one region surrounding rs12810758 (chr12: 66318027 – 66338027, GRCh37) which had posterior probability of sharing the same SNP between EWS and inguinal hernia as high as 0.80. 23 SNPs included in the analysis were located within the DNA helicase G (HMGA2) gene. An additional sensitivity analysis limiting to 0.5kb around rs12810758 showed a posterior probability of 0.81 with 3 SNPs. The top SNP rs12810758 was positively associated with inguinal hernia (p-value = 8.9x10-11, MTAG p-value = 4.5x10-11) and positively associated with EWS (p-value = 0.00089). We observed that having the alternative allele T for rs12810758 suggested lower mean values in the expression levels of HMGA2 in EWS tumors, although this difference was not significant (Figure 2).

Conclusion: We present the first genetic investigation of five hernia types (diaphragmatic, inguinal, umbilical, femoral and ventral) with EWS using two independent datasets - UKBiobank data (>60,000 individuals) and a EWS GWAS data (733 EWS cases and 1346 controls). We demonstrated, through genetic epidemiologic methods, evidence of causal positive relationships between EWS and diaphragmatic and inguinal hernias. There was no evidence of a relationship between EWS and the other three hernia types. Interestingly, we observed a locally mild but cumulatively strong relationship between diaphragmatic hernia and EWS, while we found several local genetic loci which indicated shared pathways between inguinal hernia and EWS. This analysis provides additional genetic evidence for the observed association between hernia with EWS, and also demonstrates the ability of genetic analysis to aid in unraveling the etiology of rare sarcomas.



Hernia type	nIV	F-statistics	Cochran's Q	Egger-intercept p-value	OR	95% CI	p-value
Inguinal	70	60.2	0.64	0.91	1.27	(1.01,1.59)	0.041
Diaphragmatic	24	39.2	0.98	0.33	2.26	(1.30,3.95)	0.004
Umbilical	10	52.6	0.86	0.63	1.07	(0.78,1.48)	0.66
Femoral	3	43.3	0.35	0.74	0.95	(0.73,1.23)	0.69
Ventral	4	42.3	0.59	0.36	0.96	(0.59,1.55)	0.80



(P 415) NEOADJUVANT RADIOTHERAPY MODULATES THE TUMOUR MICROENVIRONMENT OF UNDIFFERENTIATED PLEOMORPHIC SARCOMA

Samuel S. Brown, MSc; Theodore B. Verhey, PhD; Heewon Seo, PhD; Michael Monument, MD MSc FRCS(C); Sorana Morrissy, PhD, University of Calgary, Calgary, AB, Canada

Objective: Immunotherapy resistance and tumour heterogeneity in undifferentiated pleomorphic sarcoma (UPS) are driven by an immune-cold tumour microenvironment (TME) with abundant pro-tumour macrophages and low lymphocyte infiltration. While external beam radiation (EBR) can stimulate an immune-hot phenotype in certain contexts, the effects of EBR in UPS remain poorly defined. In Alberta, two neoadjuvant EBR protocols are used depending on the treatment center: 50 Grey EBR in Edmonton, and low-dose doxorubicin with 30 Grey EBR in Calgary. Both protocols are given before surgical resection to shrink the tumour mass, however, their consequences on the TME remain unknown. Here, we aim to quantify the effects of neoadjuvant EBR on both tumour and immune cells using clinical samples, a profiling strategy that accounts for distinct histological regions within tumours, and a novel application of data analysis and integration methods.

Methods: We profiled transcriptomes from 19 UPS patients, treated with either surgery only, the Edmonton, or the Calgary neoadjuvant protocols. To improve coverage of intra-tumoral TME heterogeneity, we separately sampled multiple regions within each tumour, including the invasive margin, cellular tumour near necrosis, proliferative tumour regions, and adjacent muscle tissue. Immune and tumour cell transcriptomes were collected individually using the NanoString GeoMx digital spatial profiling (DSP) platform. To validate the results we further generated global proteomics data from the same samples, and integrated these with a large external bulk RNAseq cohort.

Results: We systematically evaluated the impact of radiotherapy on surgical UPS samples with high spatial resolution across histologically-distinct tumour regions. In Aim 1, differential expression analysis identified radiation-associated genes in both tumour and TME cells with significant changes most dramatic in the tumour margin. We identified and quantified the prevalence of TME components, showing that macrophages have significantly increased abundance in the tumour margin after radiation. These results highlight the tumour margin as a

distinct ecosystem in which macrophages play a key role in the radiation response. In Aim 2, We identified gene expression programs and their usage across samples in the 3 treatment groups. Expression programs altered by radiation represented activities related to enhanced antigen presentation, upregulation of cytokine secretion, and reduced Wnt/beta-catenin signalling in the radiated samples. In Aim 3, data integration validated the existence of programs associated with an immune-hot TME, established that radiation is positively associated with this phenotype and with improved patient survival, and that the tumour margin is the site where these programs are primarily enacted. Furthermore, we confirmed a prognostic expression program related to metastatic spread and show that its usage is reduced in patients treated with radiation.

Conclusion: This study broadens our understanding of how radiation impacts UPS biology. Specifically, we identify a prognostic expression program that tumour and immune cells alter in response to high-dose radiation and show that the tumour margin is a key site where additional mechanistic insight would be of value. This work paves the way toward the identification of combination therapies that synergize with radiation to optimize the immune response in UPS.

(P 416) THE PROTEOGENOMIC SUBTYPING OF OSTEOSARCOMA

Yingqi Hua[1]; Yafei Jiang[1]; Qun Dong, n/a[2]; Binbin Hao, n/a[3]; Lei Zhao, PhD[3]; Jing Li, n/a[2]; Minjia Tan, n/a[3], (1) Department of Orthopedic Oncology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, Shanghai, Shanghai, China (People's Republic), (2) Department of Bioinformatics and Biostatistics, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China, Shanghai, Shanghai, China (People's Republic), (3) State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China, Shanghai, Shanghai, China (People's Republic)

Objective: Osteosarcoma (OS) is a prevalent malignant bone tumor that predominantly affects pediatric, adolescent, and young adult populations. Despite the significant advancements in neoadjuvant chemotherapy and surgical techniques, which have resulted in a remarkable improvement in overall survival rates to approximately 70%, there has been minimal progress in achieving substantial enhancements in the 5-year survival rates over the past four decades. Previously, we published a molecular subtyping of OS based on genomic and transcriptomic data (Nat Commun. 2022 Nov 23;13(1):7207.). However, proteomics has long been seen as a promising technique complementary to genomics for elucidating cancer biology and identifying diagnostic and predictive biomarkers.

Methods: We conducted a comprehensive proteogenomic analysis of primary OS samples derived from a cohort of 154 patients. The aim of this study was to define distinct subgroups based on proteomic profiling and evaluate their correlation with key clinical and molecular characteristics, including patient survival, tumor metastasis, chemosensitivity, and genomic profiles.

Results: The proteomic subgroups identified in this study displayed distinct molecular features related to metabolic reprogramming, microenvironment dysregulation, cell proliferation, and potential therapeutic targets. Subgroup 1, denoted as the immune subgroup (S-Im), exhibited the highest expression levels of immune, inflammatory, and stromal proteins. Subgroup 2, denoted as the metabolism dysregulated subgroup (S-Me), exhibited intermediate expression

levels of immune and proliferative proteins and showed predominant upregulation of myogenesis, fatty acid metabolism, and oxidative phosphorylation signatures. Subgroup 3, denoted as the proliferation subgroup (S-Pf), showed an increase in proliferative proteins and glycolytic activity. P4HA1, high expression specific to the S-Pf subtype, exerts inhibitory effects on the immune system while simultaneously enhancing metabolic processes. It emerges as a promising therapeutic target for the treatment of the S-Pf subtype.

Conclusion: Our comprehensive and integrated characterization of OS provides a valuable resource that enhances our understanding of the disease. These findings have the potential to guide future clinical strategies for the precise and targeted treatment of OS, ultimately improving patient outcomes.

(P 417) MULTI-OMIC CHARACTERISATION OF MOLECULAR HETEROGENEITY AND NEOADJUVANT THERAPY RESPONSE IN SYNOVIAL SARCOMAS

Madhumeeta Chadha, MSc[1]; Amani Arthur, MBChB BSc(Hons) MRCPCH[1]; Nafia Guljar, n/a[1]; Khin Thway, MD[2]; Robin L. Jones, BSc, MB BS, MRCP, MD[3]; Paul Huang, PhD[1], (1) The Institute of Cancer Research, London, England, United Kingdom, (2) The Royal Marsden NHS Foundation Trust, London, England, United Kingdom, (3) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom

Objective: Surgery is the mainstay for curative treatment of localised synovial sarcomas (SS). However, approximately 50% of patients develop recurrent disease. Neoadjuvant therapy (NAT) can improve surgical outcomes and prevent recurrence, however the biological mechanisms underlying response and resistance to NAT remains unknown. Utilising paired pre- and post-treatment specimens, we conducted an integrated proteomic and transcriptomic analysis to 1. Assess the intrinsic molecular heterogeneity within SS which could explain the range of clinical responses seen in patients and 2. Determine the molecular changes associated with NAT.

Methods: This is a single institution retrospective cohort comprising of 34 patients: 16 received NAT (n=30 specimens) and 18 did not receive NAT (treatment naive) (n=32 specimens) as a control group (Fig 1A). NAT samples comprised of biopsy (pre-treatment) and surgical (post-treatment) specimens. There was no intervening treatment between the biopsy and surgical specimen in the treatment naïve group. Paired samples refer to those cases for which matched biopsy and its corresponding excision specimen were available for profiling.
Specimens were subjected to proteomics and transcriptomics analysis. Details of specimens analysed are shown in Fig 1A. Molecular subgroups within SS were identified through consensus clustering of transcriptomic data from baseline biopsy specimens (n=24). Biological features associated with consensus clusters were determined by overrepresentation analysis (ORA) and through transcriptomic immune cell deconvolution using Microenvironment Cell Populations (MCP)-counter.
To elucidate molecular changes induced by NAT, Mfuzz soft clustering was employed to compare proteomic and transcriptomic expression patterns in paired samples. This led to the identification of subsets of proteins and genes that are associated with treatment. Altered genes and proteins were subjected to ORA.

Results: Consensus clustering of the transcriptomic data of baseline samples identified two clusters of distinct molecular profiles ($p < 0.05$) (Fig 1B). ORA showed that Cluster 2 had an

enrichment of interferon gamma signalling (Fig 1C) indicating a relatively “immune hot” molecular subgroup. This was further substantiated by immune deconvolution analysis where neutrophils, cytotoxic lymphocytes, B lineage and monocytic lineage cells showed higher levels in Cluster 2 vs Cluster 1.
For paired samples that received NAT, soft clustering of proteomics data identified 4 groups with distinct patterns (Groups A-D), each associated with proteins that changed in post-treatment compared to pre-treatment samples. As an exemplar, we highlight a subset of proteins in Group A (Fig 1D). These proteins (n=148 proteins) were downregulated in post-treatment vs pre-treatment specimens (Fig 1D left). While there was also a decrease in median expression of these proteins in some paired specimens from the treatment-naïve patients, only in the patients who received NAT was this difference statistically significant (p=0.0156 in NAT vs p=0.195 in treatment naïve). ORA showed an enrichment of ribosome and protein translation pathways in Group A, the proteosome complex in Group B and the complement cascade in Group D. A similar analysis in the transcriptomic data identified neuroactive ligand receptor interactions being enriched following NAT.

Conclusion: We show that SS is comprised of two intrinsic molecular subgroups, one which has elevated immune signalling. Moreover, we have shown that there is a consistent group of genes, proteins and biological pathways that are significantly altered in response to NAT but not in treatment naïve paired specimens. While our cohort is small and our findings hypothesis generating, this study provides important insights into the biological mechanisms associated with NAT which has potential for future applications as candidate prognostic biomarkers and identification of new drug targets for NAT.

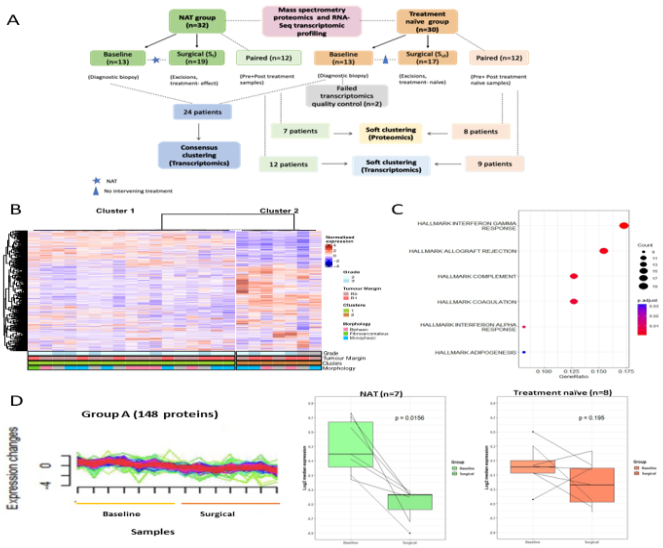


Figure1 (A) Flow diagram depicting multi-omic profiling of the cohort consisting of 62 synovial sarcoma (SS) samples split across NAT group and treatment naïve group. Each group consists of baseline, surgical and paired samples where S_1 and S_2 are treated and untreated surgical specimens. Samples used for downstream analysis; consensus clustering and soft clustering and those failing quality control are also depicted. **(B)** Heatmap depicting n=2054 genes with variance>0.5 for 24 baseline SS samples with K=2 identified through consensus clustering with annotation bars showing grade, tumour margins, clusters and grade at the bottom of the heatmap. **(C)** Dot plot depicting Hallmark pathways enriched in Cluster 2 of baseline SS through overrepresentation analysis. **(D)** Soft clustering depicting expression pattern of 148 proteins in Group A that decrease in surgical samples (x axis ;samples and y axis ;normalised expression) (left) alongside boxplots depicting median log2 expression of the proteins in NAT and treatment-naïve groups determined by Wilcoxon paired rank test (right).

(P 418) GENOMIC AND IMMUNOLOGICAL PROFILING OF PRIMARY AND DISTANT METASTASES OF SOFT TISSUE SARCOMAS

Mariella SPALATO-CERUSO, MD[1]; Stephanie VERBEKE, PhD[1]; Vanessa CHAIRE, n/a[1]; Aurélien BOURDON, n/a[1]; Veronica Yanina VALVERDE, n/a[1]; Alban BESSEDE, PhD[2]; Jean-Philippe GUEGAN, PhD[2]; Antoine Italiano, MD,PhD[1], (1) Institut Bergonié, Bordeaux, Aquitaine, France, (2) Explicyte, Bordeaux, Aquitaine, France

Objective: Soft tissue sarcomas (STS) are a diverse group of rare tumors that exhibit metastasis in approximately 50% of patients. Unfortunately, the prognosis for metastatic STS is typically fatal, with an average survival of only 20 months. Throughout the metastatic process, tumor cells undergo mutational changes and encounter selective treatment pressures, resulting in altered molecular characteristics. However, the genetic and immunological profiles of these challenging tumors and sarcoma metastases remain poorly understood.

Methods: We performed high throughput analysis of a cohort of 70 patients with soft tissue sarcoma, each with paired primary tumors and matched metastases. The histological subtypes included in the study are undifferentiated pleomorphic sarcoma (UPS) [n = 20], leiomyosarcoma (LMS) [n = 17], liposarcoma (LPS) [n = 19], and other rare histologies [n = 14]. Our primary objective is to compare the transcriptomic profiles of primary and metastatic tumors by analyzing the RNAseq data from our patient cohort. Additionally, we employ spatial biology profiling using the GeoMx® Digital Spatial Profiler system (DSP) to perform quantitative, high-plex analysis of mRNA expression within spatially defined regions of the primary tumors and metastases. Through these analyses, we aim to identify differentially expressed genes (both upregulated and downregulated) between primary and metastatic tumors, which may shed light on candidate genes or pathways involved in the metastatic process based on co-expression transcriptional networks.

Results: Analysis of the UPS sub-cohort revealed an overexpression of inflammatory pathways and hypoxia in metastatic samples compared to paired primary (Figure 1C). Specifically, two genes, ADORA2B and SERPINE1, exhibited higher expression in metastases compared to primary tumors (Figure 1A and 1B). These genes potentially play a role in the metastatic process, immune infiltration, and angiogenesis. Additionally, we observed an overexpression of VEGF in the stromal regions of metastases (Figure 1B). Notably, these genes have been associated with worse overall survival in GEPIA database analyses (Figure 2A and 2B). Furthermore, our spatial transcriptomic profiling revealed an overexpression of TGFbeta in metastases and IDO1 in primary tumors, indicating immunological differences between the two tumor types (Figure 1B). Multiplex immunohistochemistry analyses allowed to confirm these findings.

Conclusion: This study represents the largest transcriptomic profiling of paired primary and metastatic STS tumors. Our findings provide valuable insights into genes potentially involved in the metastatic process in UPS, one of the most common and undifferentiated subtypes of STS. Results from the other cohorts and of functional experiments will be presented at the meeting.

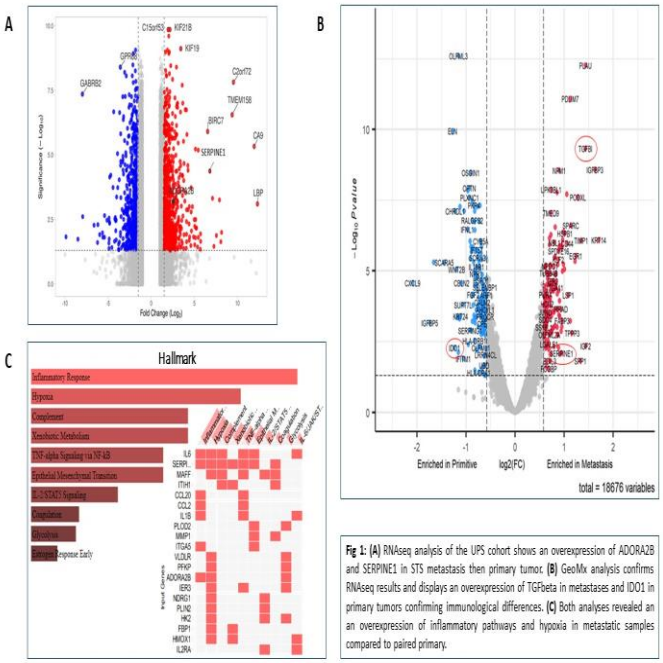


Fig 1: **(A)** RNAseq analysis of the UPS cohort shows an overexpression of ADORA2B and SERPINE1 in STS metastasis then primary tumor. **(B)** GeoMx analysis confirms RNAseq results and displays an overexpression of TGFbeta in metastases and IDO1 in primary tumors confirming immunological differences. **(C)** Both analyses revealed an an overexpression of inflammatory pathways and hypoxia in metastatic samples compared to paired primary.

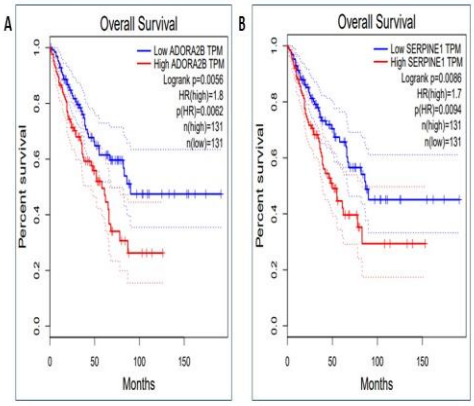


Fig 2: GEPIA database analyses showing a worse overall survival in soft tissues sarcomas overexpressing ADORA2B **(A)** and SERPINE1 **(B)**, suggesting a prognostic value of these two genes that we found overexpressed in metastases.

(P 419) AGE ADJUSTED TRENDS IN PATIENTS WITH SYNOVIAL SARCOMA

Amr Aly, MBBCh[1]; Asmaa.A. Abdeltawab, MBBCh, MD[2]; Ahmed Eldesoky, MBBCh, MD[3]; Mohamed Kelany, MBBCh, MD[4], (1) Faculty of medicines Suez Canal University, Ismailia, Al Isma'iliyah, Egypt, (2) Faculty of medicines Asyut University, Asyut, Asyut, Egypt, (3) Faculty of medicines Mansoura University, Al Mansoura, Ad Daqahliyah, Egypt, (4) Faculty of medicines Ain Shams University, Cairo, Al Qahirah, Egypt

Objective: Synovial sarcoma is an uncommon malignant tumor that predominantly affects muscles and ligaments. We have a very limited data regarding the trends and incidence rates of this rare type of sarcoma due to its scarcity which lead to controversy in regards of its screening and treatment guidelines. So, this study aims to add more evidence and data about this rare type.

Methods: Data of 2,872 patients was extracted from the Surveillance, Epidemiological, and End Results (SEER) database diagnosed from 2000-2019. The histopathological classification was according to the WHO ICD 0-3. We analyzed Age-Adjusted Trends and Age-adjusted incidence rates. Rates are per 100,000 and age-adjusted to the 2000 US Std Population (19 age groups - Census P25-1130) standard; Confidence intervals are 95% for rates (Tiwari mod) and trends. Percent changes were calculated using 1 year for each end point; APCs were calculated using weighted least squares method. All data are from cases diagnosed between 2000-2019.

Results: The overall incidence rate was 0.17. The age-adjusted trend analysis showed that annual percentage change (APC) was 0.1 (95%CI -0.5 to 0.7) from the year 2000 to 2019 and the percent change (PC) was 5.1. This increase occurred across all ages, races, sexes and subtypes. With stratification by histopathology, NOS, spindle cell, biphasic cell and epithelioid cell synovial sarcoma PCs were -11.5, 108.4, -23.6, and -78.4, respectively and the APCs were 0.1 (95%CI -0.9 to 1.1), 1.0 (95%CI-0.6 to 2.5), -1.6 (95%CI -3.0 to -0.1) and it was missing for epithelioid cell. Males showed higher PC of 11,7 (APC: -0.2 95%CI -1.1 to 0.7) compared to females; -2.0 (APC: 0.4 95%CI -0.5 to 1.3). With stratification by age groups, age groups 0-14, 15-29, 30-44, 45-59 and 60+ showed PCs of -5.9, -18.3, -8.6, 47.5 and 50.6 respectively and APCs of -0.6 (95%CI -3.6 to 2.5), -1.1 (95%CI -2.0 to -0.1), 0.4 (95%CI -0.8 to 1.6), 0.7 (95%CI -0.8 to 2.3) and 0.9 (95%CI -0.9 to 2.6) respectively.

Conclusion: Synovial sarcoma showed an increase in its overall incidence rate. However, with subtyping of Synovial sarcoma, spindle cell subtype showed the main increase while NOS, epithelioid cell and biphasic cell subtypes showed a decline in their incidence rates. Synovial sarcoma showed rising incidence rates in Males while for Females, it was declining. Only age groups over 45 showed an increase in their incidence rates. This increase in the incidence rates of synovial sarcoma warrants the need of the development of screening guidelines. Also, we recommend focusing on males and patients above 45 as they have showed the most rise in their incidence rates.

(P 420) COMPREHENSIVE MOLECULAR PROFILING AND TUMOR MICROENVIRONMENT CHARACTERISTICS OF RADIATION-INDUCED SARCOMA.

Marina Voropaeva, MD; Konstantin Zirov, MD; Irina Zhuk, MD; Aida Aukhadieva, MD; Anastasia Makarova, MD; Aleksei E. Shevkoplias; Krystle Nomie, PhD; Anna Love, PhD; Nathan Fowler, MD; Alexander Bagaev, PhD; Lev Bedniagin, MD, BostonGene, Corp., Waltham, MA

Objective: A late-onset complication of radiotherapy (RT), radiation-induced sarcoma (RIS) is a rare tumor associated with chemotherapy resistance and poor prognosis. Although immune checkpoint inhibitors (ICI) are recommended for several soft tissue tumors, their efficacy has not been demonstrated in RIS. Therefore, identifying effective therapies for RIS remains an unmet need. Comprehensive molecular testing including whole exome sequencing (WES) and RNA-seq was performed to characterize the tumor microenvironment (TME) composition and uncover potential genomic drivers of RIS to guide therapeutic decision-making.

Methods: Sarcoma samples and clinical data from an internal database were screened to match RIS criteria, including a history of prior RT, a period of latency after RT (>2 years), and histologic confirmation of sarcoma. WES and RNA-seq were performed as a part of the BostonGene Tumor Portrait® test for 9 RIS samples. Retrospective analysis of the test results revealed transcriptomic and genomic alterations associated with RIS, and samples were assigned RNA-based immune-enriched (IE), immune-enriched, nonfibrotic (IE/M), fibrotic (F), or desert (D) TME subtypes. which we previously described in Bagaev et al.

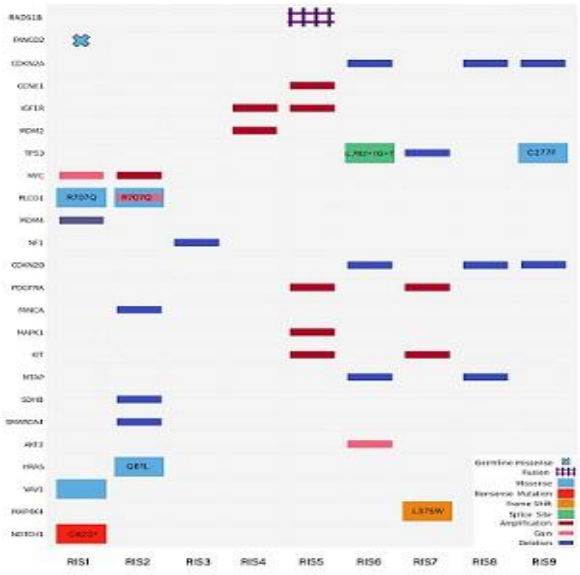
Results: Histological subtypes included angiosarcoma (n = 3), sarcoma not otherwise specified (NOS, n = 3), osteosarcoma (n = 1), and malignant peripheral nerve sheath tumor (n = 2, Table 1). The median duration between the initial RT and current sarcoma was 10 years (3-33 years). The cohort included 5 women and 4 men with a median age of 53 years (34-80 years) at the time of RT. The most frequent primary tumor was breast cancer (n = 5).
During transcriptomic analysis, five samples (56%) contained IE TME subtypes characterized by low levels of stromal and fibrotic elements and high levels of immune infiltrate, including cytotoxic effector cells. The remaining four patients (44%) had fibrotic (F) TMEs with minimal immune infiltration and high content of stromal components.
Six patients were treated with systemic therapy prior to molecular testing. All six patients with treatment data received chemotherapy, and of these patients, additional therapies included single-agent pazopanib (n = 2), combination chemotherapy plus ICI (n = 2). Response data were available for two patients with IE TMEs who received ICIs with clinically positive responses. Of these two patients, one exhibited stable disease during the 7 months of advanced disease treatment, and the other patient received ICI and taxane neoadjuvant therapy for 4 months prior to surgery.
WES was performed to assess for somatic alterations that could serve as potential drivers or targets, such as genes associated with oncogenic pathways or DNA damage response (Figure 1). Transcriptomic analysis identified high MET expression in 6 patients and high CD274 (PD-L1) expression in 4 patients. Low tumor mutational burden and microsatellite stability were observed in all samples. Potential clinical trials were matched for all 9 RIS cases based on these molecular findings.

Conclusion: BostonGene's Tumor Portrait® test identified clinically relevant findings for 9 RIS patients, who received integrated WES and RNA-seq testing. Over 50% of RIS patients had an IE TME subtype, which indicated the probability of a favorable response to ICI. Integrated WES and RNA-seq analysis can be used to select targeted molecular-based therapies and identify

clinical trials for RIS patients.

Table 1: Clinical features and tumor microenvironment subtypes in radiation-induced sarcoma (RIS) patients

		Primary tumor		Secondary tumor		
	Sex	Histology	RT was performed (year)	Histology	Date of onset (year)	TME subtype
RIS1	F	Breast cancer	2010	Angiosarcoma	2020	Immune-enriched
RIS2	F	Breast cancer	2018	Angiosarcoma	2021	Immune-enriched
RIS3	M	Oropharyngeal squamous cell carcinoma	2017	Angiosarcoma	2022	Immune-enriched
RIS4	M	Testicular cancer	1991	Malignant peripheral nerve sheath tumor	2019	Immune-enriched
RIS5	F	Breast cancer	2013	Osteosarcoma	2023	Fibrotic
RIS6	F	Breast cancer	1990	Sarcoma NOS	2023	Immune-enriched
RIS7	F	Breast cancer	2009	Sarcoma NOS	2021	Fibrotic
RIS8	M	Prostate cancer	2000	Malignant peripheral nerve sheath tumor	2015	Fibrotic
RIS9	M	Myxoid liposarcoma	2018	Sarcoma NOS	2022	Fibrotic



(P 421) **USING TRANSCRIPTOMICS TO IDENTIFY LEIOMYOSARCOMA PATIENTS AT RISK OF METASTASIS**

Adam M. Fontebasso, MD, PhD[1]; Joshua Nash[2]; Yael Babichev, PhD[3]; Hagit Peretz Soroka, Ph.D.[4]; Abha Gupta, MD MSc[5]; Brendan C. Dickson, MD[6]; Elizabeth Demicco, MD, PhD[6]; Adam Shlien, PhD, FCCMG[2]; Rebecca Gladdy, MD, PhD[7], (1) University of Toronto, Toronto,

ON, Canada, (2) The Hospital for Sick Children, Toronto, ON, Canada, (3) Sinai Healthcare System, Toronto, ON, Canada, (4) Princess Margaret Cancer Centre / The Hospital For Sick Children, Toronto, ON, Canada, (5) Princess Margaret Cancer Center, Toronto, ON, Canada, (6) Sinai Health System, Toronto, ON, Canada, (7) Mount Sinai Hospital, Sinai Health System, and Princess Margaret Cancer Centre Toronto, ON, Canada Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada University of Toronto, Toronto, ON, Toronto, ON, Canada

Objective: OBJECTIVE: Leiomyosarcoma (LMS) is a rare, aggressive mesenchymal malignancy and represents one of the more common forms of soft tissue sarcoma (STS). Although distant disease failure constitutes the primary feature governing patient outcome, there are no universally accepted biomarkers which can identify patients at high risk for metastatic progression. As the role for adjuvant systemic therapy remains controversial in this disease, we hypothesize that enhanced characterization of LMS molecular subgroups has the potential to better define clinically important biomarkers to tailor more biologically-informed treatments. As such our objective for this study is to robustly identify and characterize molecular subgroups of LMS and translate their utility to the clinical setting.

Methods: METHODS: After Research Ethics Board approval and appropriate Materials Transfer Agreement, we recruited a large cohort of n = 195 LMS tumors and n = 17 leiomyomas with transcriptome data from the Toronto Sarcoma Program, The Cancer Genome Atlas (TCGA), Clinical Sequencing Evidence-Generating Research (CSER) and collaboration with the European Union. We complemented this with clinical data from the respective sources as well as under the Canadian Sarcoma Research and Clinical Collaboration (CanSARCC) consortium agreement. We performed transcriptome analysis using an established locally-developed, previously validated bioinformatic pipeline called RACCOON (Resolution-Adaptive Coarse-to-fine Clusters OptimizatiON), a multilevel RNA-seq unsupervised hierarchical clustering approach to identify molecular subgroups with all LMS tumors, leiomyomas as well as previously published large-scale transcriptome data (n = 13,313 transcriptomes, Comitani et al, Nature Medicine 2023). Translational implications of these molecular subgroups were analyzed through annotation with demographic and clinical variables for survival analysis utilizing Kaplan-Meier methodology and analyzed using GraphPad Prism software (GraphPad Prism 8, Boston USA).

Results: RESULTS: Transcriptome analysis of the dataset utilizing RACCOON robustly identified three major subgroups of LMS with further multilevel coarse-to-fine clustering delineating further heterogeneity notably of subtype 2 into finer clusters (n = 7). Kaplan-Meier survival analysis comparing clusters identified statistically significantly worse event-free survival associated with subtype 2a ($P < 0.05$, Gehan-Breslow-Wilcoxon test), but no differences in overall survival. The proportion of patients who developed metastatic disease differed between subgroups, with approximately 75% (12/16) subtype 2a patients developing metastases.

Conclusion: CONCLUSIONS: Large-scale transcriptomics can delineate biologically distinct molecular subgroups of LMS and in particular can help to identify high-risk subgroups with distinct propensity for the development of metastases. Collaborative efforts in the LMS community will aid in further refinement and validation, which is necessary prior to clinical usage.

(P 422) CLINICAL UTILITY OF WHOLE GENOME SEQUENCING FOR THE IDENTIFICATION OF ACTIONABLE GENETIC ALTERATIONS IN SARCOMA PATIENTS: A RETROSPECTIVE COHORT ANALYSIS

Suha Abdulla, MBBS MRCP MRes PgCert (MedEd); Midhat Ahmed, MBBS BSc; Rachael Windsor, BSc Hons MBBS Hons MRCPCH MSc MDRes; Georgina Elizabeth Wood, MBBS MRCP BSc(hons) MSc PhD, University College London Hospital, London, England, United Kingdom

Objective: Sarcomas are rare, mesenchymal tumours exhibiting significant heterogeneity, with more than 100 subtypes recognised by the WHO classification (1). Following the 100,000 genomes project, across England, all patients diagnosed with sarcoma are entitled to whole genome sequencing (WGS), which is approved and funded by NHSE since 2018. WGS is a useful tool for refining diagnoses and identifying novel genomic alterations (2). There are ongoing challenges of optimising uptake of testing, delivery of the service within the NHS and utilising results to enhance patient care including access to personalised therapies and clinical trials. The clinical utility of WGS at diagnosis for paediatric, teenage and young adult (TYA) and adult sarcoma patients as well as its impact on outcomes requires further elucidation.

Methods: We retrospectively analysed clinical data for all new or relapsed sarcoma diagnoses in paediatric, TYA and adult patients consented for WGS from May 2021 to September 2022 within the London Sarcoma Service (LSS). Patients were identified using a local electronic healthcare record system. Timing from consent and sample collection to acquisition of results and patients being informed was analysed, along with genetic findings and genomic tumour advisory board (GTAB) outcomes.

Results: Clinical records of 45 paediatric/TYA and 43 adult sarcoma patients that had consented to WGS were identified. From these, WGS was completed and results available for 25 paediatric/TYA and 5 adult patients. 24 patients were informed of their results (paediatric/TYA 22, adult 2). Median time from patient consent to being informed of results was 13 weeks (range 6 – 67).

In our paediatric/TYA cohort, the histological subtypes were osteosarcoma (OS) (n=18), Ewing sarcoma (n= 5), rhabdomyosarcoma (n=1) and malignant peripheral nerve sheath tumour (n=1). The median age was 15 years (range 6-22). WGS identified two patients with germline variants – MLH1 variant and TP53 variant (both OS). Both patients were referred to clinical genetics services and Lynch syndrome and Li-Fraumeni syndrome were confirmed. The patient with Lynch syndrome subsequently received compassionate access immunotherapy, based on this result. The most frequent somatic genetic variants were TP53 (n=12), RB1 (n=8), gain of 8q (n=8), gain of MYC (n=6), gain of 17p (n=5), CDKN2A (n=4) and aneuploidy (n=4). In one paediatric patient with high grade osteoblastic osteosarcoma, a novel fusion gene, PSMC1::NFATC2 was identified.

In the adult cohort, the histological subtypes were chordoma (n=1), OS (n=1), small round cell sarcoma (n=1), myxofibrosarcoma (n=1) and pleomorphic liposarcoma (PLS) (n=1). The median age was 72 years (range 35-75). WGS for one patient with PLS was resulted after they had died. The most frequent somatic genetic variants were FGF14, TP53, RB1, CDKN2A, ATRX and PTEN.

Overall, 47% of patients had at least one AMP tier IA variant, 3% AMP tier IIC, 16% AMP tier IID and 16% tier 3 variants. None of these variants were actionable for referral to clinical trials.

Conclusion: WGS has shown clinical utility in identifying pathogenic germline variants. Based

on germline MLH1 variant confirmation, one patient received compassionate access immunotherapy. Although almost 50% of patients had a clinically relevant tumour genetic alteration (eg. TP53 variant), these remain non-actionable for sarcoma patients and therefore no patients were referred to clinical trials based on these results. One novel somatic gene fusion was identified.

Long intervals from sampling to issuing of results remain a challenge. Documentation being held across different trusts created gaps in patient data and limited our analysis. To enhance understanding and communication, in October 2022 our local pathway protocol formalized that all WGS results are discussed at the LSS genomic tumour advisory board meeting (GTAB).

Our next steps involve - optimisation of pathways to capture all eligible patients; reduction of timescales for results; utilisation of electronic healthcare records to streamline documentation; establishing a WGS database with the future aim of collaborating with national and international centres to facilitate improved access to clinical trials for sarcoma patients.

(P 423) **PRIMARY GLIOSARCOMA; IMPACT OF ADJUVANT CHEMO(RADIO)THERAPY ON SURVIVAL OUTCOME**

Amr Aly, MBBCh; Asmaa Ellaithy, MBBCh, Faculty of medicines Suez Canal University, Ismailia, Al Isma'iliyah, Egypt

Objective: Gliosarcoma is a rare very aggressive tumor affecting the brain and the central nervous system. It has high mortality rates. Multimodality treatment is recommended including Subtotal or total tumor resection with adjuvant chemotherapy or radiotherapy. The impact of adjuvant therapy is controversial So, this study aims to assess the impact of adjuvant therapy on survival outcome.

Methods: We extracted the data of 780 patients from Surveillance, Epidemiological, and End Results (SEER) database diagnosed from 2000-2019. We assigned the patients into 4 subgroups; surgery with no adjuvant therapy, adjuvant chemotherapy, adjuvant radiotherapy, adjuvant combined chemoradiotherapy. Surgeries included were: Tumor destruction, Local excision of the tumor both total and subtotal excision, Radical total gross resection of tumor, and gross total resection of lobe of brain (lobectomy). We used SPSS 25 for data analysis, performed Kaplan-Meier and log rank test for survival analysis.

Results: The overall 3 years and 5-year survival is 10.3% and 5.9%. the 5-year relative survival for adjuvant chemotherapy, adjuvant chemoradiotherapy, adjuvant radiotherapy and surgery with no systemic therapy (10.2%,7.9%,2.2 % and 0.8% respectively; P< 0.0001). Out of 780 patients, 61.8% were males while females represented 38.2%. Kaplan Meier analysis stratified by gender showed significant impact (P< 0.0001).

Conclusion: Gliosarcoma has poor 3-year and 5-year Survival outcome in all subgroups. Adjuvant chemotherapy shows quite similar 5-year survival outcome for combined adjuvant chemoradiotherapy while adjuvant radiotherapy and surgery with no systemic therapy had the worst survival outcome. The current guidelines recommend combined adjuvant modality for gliosarcoma management, however our results find no survival benefit for adjuvant radiotherapy. Therefore, we can avoid unnecessary serious side effects of radiotherapy for better post treatment quality of life.

(P 424) INTERNAL TANDEM DUPLICATION OF BCOR (BCOR-ITD) ALTERS THE TUMOR EPIGENOME

Maureen J. O'Sullivan, MBBCh, MD, FRCPath[1]; Ismail Elgenaidi, PhD[2]; Christopher Keane, BSc[2]; David Reck, BSc[2]; Ibrahim Syed, PhD[2]; Colin Kenny, PhD[3]; Fiona M. Roche, PhD[4]; Gerard Cagney, PhD[5]; Andrew Flaus, PhD[6]; Karsten Hokamp, PhD[2], (1) Children's Health Ireland & Trinity College, Dublin, IRELAND, Dublin, Dublin, Ireland, (2) TCD, Dublin, Dublin, Ireland, (3) University of Iowa, Iowa, IA, (4) TCD, Dublin, N/A, Ireland, (5) University College Dublin, Dublin, Dublin, Ireland, (6) University College Galway, Galway, Galway, Ireland

Objective: In this work we aim to integrate RNA expression, DNA methylation, ChIP Seq, size exclusion chromatography and IP-mass spec data from our cell line models to elucidate the oncogenic mechanism in BCOR family tumors.

Methods: We generated an inducible cell line model of YWHAE::NUTM2 fusion tumors, and further cell line models stably expressing BCOR-wild type versus three of the internal tandem duplications previously described by our group in a large series of clear cell sarcomas of kidney to include the commonest ITD and an observed triplication event also. IP-mass spectrometry was carried out comparing ITD versus wild type-BCOR-expressing constructs along with size exclusion chromatography studies also. DNA methylation analyses were similarly carried out on BCOR ITD versus wild-type cell lines as well as and ChIPSeq. RNA sequencing of all cell lines including BCOR and YWHAE::NUTM2 construct-bearing lines was completed also.

Results: Reassuringly, the cell line models show gene expression changes including many previously reported in BCOR-family tumor samples in the literature. Three lines of evidence, including IP-mass spectrometry, molecular dynamic studies and size exclusion chromatography all support the hypothesis that BCOR-ITD leads to non-assembly/disaggregation of the PRC1.1, NOT through failed interaction of BCOR-ITD with PCGF1, its direct interaction partner subunit, as has been reported in leukemias in which there is typically a truncating mutation of BCOR, rather than ITD, and resulting in loss of BCOR's PUFD domain required for the interaction with PCGF1. Instead, this BCOR-PCGF1 interaction and indeed subsequent heterotrimerisation with KDM2B persist in the context of BCOR-ITD, but with separation of the enzymatic RING1B/RYBP subunits from the DNA-binding BCOR/PCGF1/KDM2B subcomplex. We propose a novel and intriguing mechanism for the 'disintegration/non-assembly' of the complex in this way, which relies on a further PRC1.1 protein undergoing conformational change in this context. We show by ChIPSeq and RNA profiling how the histone modification by PRC1.1 is altered in the ITD context with both direct and indirect consequences on gene expression in these cell lines.

Conclusion: A novel mechanism of oncogenesis is revealed, which differs importantly from the loss of function truncating mutations identified in BCOR in the leukemia setting. Further exploration is expected to pave the way for novel therapeutic approaches.

Sarcoma of the Year: Small Round Blue Cell Tumor

(P 425) CHARACTERIZATION OF RHABDOMYOSARCOMA WITH PAX7::FOXO1 FUSIONS: A REPORT FROM THE SOFT TISSUE COMMITTEE OF THE CHILDREN'S ONCOLOGY GROUP

Archana Shenoy, MD[1]; Wei Xue, PhD[2]; Amira Qumseya, n/a[3]; Abha Gupta, MD MSc[4]; Timothy B. Lautz, MD[5]; Suzanne Wolden, MD[6]; Jonathan Metts, MD[7]; Shalini Reshmi, PhD[8]; Elizabeth Wagner[9]; Rajkumar Venkatramani, MD[10]; Sonja Chen, MD[1]; Michael Arnold, MD PhD[11]; Erin Rudzinski, MD[12]; Lisa a. Teot, MD[13], (1) Nationwide Children's Hospital/The Ohio State University College of Medicine, Columbus, OH, (2) University of Florida, Gainsville, FL, (3) University of Florida, Gainesville, FL, (4) Princess Margaret Cancer Center, Toronto, ON, Canada, (5) Lurie Children's Hospital/Northwestern University, Chicago, IL, (6) Memorial Sloan Kettering Cancer Center, New York, NY, (7) Moffitt Cancer Center and Johns Hopkins All Children's Hospital, Tampa, FL, (8) Nationwide Children's Hospital Institute for Genomic Medicine/The Ohio State University College of Medicine, Columbus, OH, (9) Nationwide Children's Hospital, Columbus, OH, (10) Baylor College of Medicine / Texas Children's Hospital, Houston, TX, (11) Children's Hospital of Colorado/University of Colorado, Aurora, CO, (12) Seattle Children's Hospital/University of Washington, Seattle, WA, (13) Boston Children's Hospital and Harvard Medical School, Boston, MA

Objective: Alveolar rhabdomyosarcoma (ARMS) is characterized by specific morphologic features, including round cell morphology arranged in sheets or in nests separated by delicate fibrous septa, as well as molecular features, including the presence of a PAX3 or PAX7::FOXO1 gene fusion in 80-90% of cases. The pathologic characteristics ARMS with PAX7 alterations are not well established, however. ARST1431, the Children’s Oncology Group intermediate risk rhabdomyosarcoma (RMS) clinical trial (supported by Pfizer), included central pathology review as well as identification of FOXO1 fusion partner for all patients with FOXO1 positive RMS, allowing detailed analysis of the morphology for PAX7::FOXO1 positive RMS.

Methods: Central pathology review was conducted by five pathologists, with consensus agreement on histologic subtype and immunohistochemical staining pattern including myogenin expression. Diagnoses were made using the 5th edition World Health Organization Classification for Soft Tissue and Bone Tumours, with the exception of mixed RMS which was defined as discrete, circumscribed regions of both alveolar and embryonal morphology within a single tumor. Institutional confirmation of FOXO1 fusion status was required for all patients enrolled on ARST1431, and all FOXO1 fusion positive cases underwent centralized testing for PAX3 or PAX7 rearrangements using fluorescent in-situ hybridization (FISH).

Results: Central pathology review was conducted for 306 patients enrolled on ARST1431. Of these, 58.5% had embryonal histology, 28.8% were histologic ARMS, 7.5% were spindle cell/sclerosing RMS and 1.6% were mixed embryonal/alveolar morphology, with the remaining 3.6% being insufficient for histologic classification. There were 27.1% fusion positive RMS, of

which 16% (n=13) harbored a PAX7::FOXO1 gene fusion. Nine patients were female and four male, ages ranging from 11 months to 22 years (Median: 3 years). These tumors occurred in the extremity (n = 5), trunk (n = 4) and head/neck (n = 4) locations. Eight tumors (69%) demonstrated alveolar morphology (7 classic and 1 solid pattern) and four (31%) had a mixed alveolar and embryonal morphology. One case had insufficient tissue for histologic classification. Notably, only one other mixed RMS was identified in the ARST1431 cohort; this tumor harbored a FOXO1 rearrangement with amplification of the FOXO1 gene but a fusion partner was unable to be confirmed on central FISH analysis. Rhabdomyoblastic differentiation was noted in 77% (10/13) of RMS with PAX7::FOXO1 in comparison to 30% (19/64) of RMS with PAX3::FOXO1 fusions. Myogenin expression was strong and diffuse in all tumors with alveolar morphology, although decreased expression was seen in areas of embryonal morphology in mixed RMS cases.

Conclusion: RMS with PAX7 alterations predominantly demonstrate alveolar morphology; however, a subset of tumors demonstrate true mixed alveolar/embryonal morphology. Mixed embryonal and alveolar morphology is a potential morphologic surrogate for suspecting an RMS with PAX7 alterations.

Patient no.	Age	Gender	Site	Histologic subtype	If mixed, composition of ARMS vs. ERMS	Rhabdomyoblastic differentiation
1	2 years	M	Extremity	ARMS#, classic		Present
2	1 year	F	Trunk	RMS^, mixed	30% ARMS, 70% ERMS*	Present
3	11 months	M	Trunk	RMS, Mixed	75% ARMS, 25% ERMS	Present
4	9 years	M	Trunk	RMS, mixed	90% ARMS, 10% ERMS	Absent
5	3 years	F	Trunk	RMS, mixed	80% ARMS, 20% ERMS	Present
6	3 years	F	Head and Neck	ARMS, solid		Present
7	1 year	F	Head and Neck	ARMS, classic		Absent
8	6 years	F	Extremity	ARMS, classic		Absent
9	3 years	F	Extremity	ARMS, classic		Absent
10	22 years	F	Head and Neck	ARMS, classic		Present
11	13 years	F	Head and Neck	RMS, not otherwise specified		Present
12	4 years	M	Extremity	ARMS, classic		Present
13	12 years	F	Extremity	ARMS, classic		Present

ARMS - Alveolar Rhabdomyosarcoma

^ RMS - Rhabdomyosarcoma

* ERMS - Embryonal rhabdomyosarcoma

(P 426) MULTI-OMIC ANALYSIS OF DESMOPLASTIC SMALL ROUND CELL TUMORS REVEAL DISTINCT LINEAGES

Danh D. Truong, PhD[1]; Emre Arslan, PhD[2]; Veena Kochat, PhD[1]; Sandhya Krishnan, MS[1]; Clement Nana Agyemang, MS[1]; Margarita Divenko, BS[3]; Davis R. Ingram, BS[1]; Rossana Lazcano Segura, MD[1]; Akshay Basi, BS[1]; Javier Gomez, BS[4]; Hannah Beird, PhD[5]; Chia-Chin wu, PhD[1]; Jared Burks, PhD[1]; P Andrew Futreal, PhD[1]; Alexander J. Lazar, MD, PhD[1]; Ravin

Ratan, MD[1]; Najat C. Daw, MD[4]; Andrea Hayes-Dixon, MD[6]; Kunal Rai, PhD[1]; Joseph A. Ludwig, IV, Ph.D.[7], (1) The University of Texas MD Anderson Cancer Center, Houston, TX, (2) The University of Texas MD Anderson Cancer Center, Melrose, MA, (3) Baylor College of Medicine, Houston, TX, (4) MD Anderson Cancer Center, Houston, TX, (5) Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA, Houston, TX, (6) Howard, Washington, DC, (7) Department of Sarcoma Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX

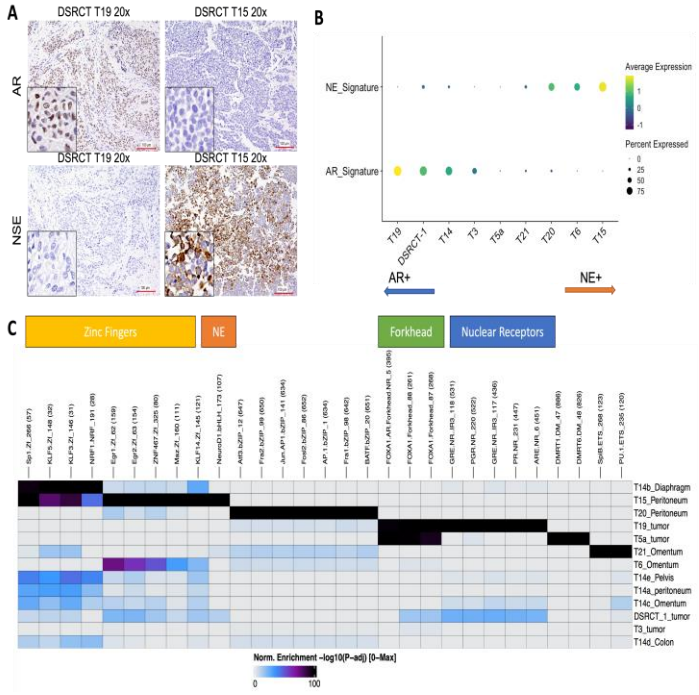
Objective: Desmoplastic small round cell tumor (DSRCT) is a rare and usually incurable aggressive sarcoma subtype. All malignant cells harbor a pathognomonic EWS-WT1 fusion protein (FP), but FP-targeted agents are nonexistent, and less than 20% of patients survive beyond five years. Lacking the availability of FP-targeted drugs, we seek to better understand how the FP regulates tumor cells to promote the characteristic multilineage phenotypes of epithelial and neuroendocrine (NE) cell fates to discover a therapeutic opportunity.
Our data showed that a subset of DSRCT cells (mostly of epithelial lineage) highly express the androgen receptor (AR), which is the pivotal epigenetic driver of prostate cancer (PCa). We showed that DSRCT depended on AR signaling by targeting AR with enzalutamide, a nonsteroidal antiandrogen, where we observed delayed tumor growth response in a DSRCT xenograft and a patient-derived xenograft. Our team also revealed that the AR cis-elements were enriched for specific TF motifs, including those from forkhead box (FOXA1), response elements for nuclear receptors (ARE, GRE, PGR), and zinc finger proteins (WT1, EGR1). Given the observation of the WT1 (the C-terminal partner of the pathognomonic fusion protein) binding motif, we hypothesized that the FP, FOXA1, and nuclear receptors interact together at the regulatory sites of AR, driving the epithelial and NE lineages in DSRCT.
Herein, we share the results of gene expression and chromatin accessibility analyses to explore fundamental mechanisms that may be driving epithelial and NE biology in DSRCT.

Methods: To assess gene expression and chromatin accessibility, we performed Chromium Single Cell Multiome ATAC + Gene Expression on thirteen fresh-frozen specimens from nine patients. We delineated malignant cells from normal cells using EWS-WT1 gene signatures, copy number variation, and CytoTRACE analysis. CytoTRACE is a computational tool designed to predict differentiation states. We assessed multilineage expression using epithelial and neuroendocrine signatures. Using the ATAC-seq data, we observed significant peaks and motifs associated with different lineages. To assess spatial heterogeneity and proteomic profile, we used the Lunaphore COMET, which enables up multiplex immunofluorescent imaging of slides.

Results: We profiled 85,640 nuclei from nine DSRCT patients using single-nucleus RNA-sequencing and ATAC-sequencing from fresh-frozen tissue. We found a subset of DSRCT patients with low or negative expression of AR that expressed partial NE reprogramming, akin to NE prostate cancer (NEPC, Panel A). One subset (NE: T6, T15, T20, T21, DSRCT-1) exhibited NE markers (SYP, ENO2, CHG, FOXA2, ASCL1, and SOX2), while a separate subset (Epithelial: T14, T19, T3) expressed epithelial markers (MUC1, MUC6, KRT18, KRT23, CDH1). T5a appeared to exhibit both sets of markers – implying a hybrid phenotype or perhaps poorly differentiated phenotype. Additionally, COMET data showed that AR and cytokeratin were anti-correlated with neural specific enolase, an NE marker. This suggests that DSRCT could be classified into three subtypes: 1) Epithelial/AR-positive, 2) NE-positive, and 3) hybrid AR/NE (Panel B).
Given the heterogeneous expression programs in DSRCT, we asked if these states were related to differentiation and plasticity. We used CytoTRACE to predict differentiation states. Expectedly, normal cells (i.e., fibroblasts, and immune cells) were the most differentiated.

Neoplastic cells, identified by EWS-WT1 gene targets and copy number variations, had lower differentiation scores, indicative of a dedifferentiated phenotype. The NE subtype had the lowest differentiation score indicative of more plastic capacity, which has previously been implicated in NEPC.
Single-cell ATAC-seq data revealed enriched motifs associated with different DSRCT subtypes. The WT1 motif was enriched in clusters identified as malignant cells. Epithelial/AR-positive subtypes showed enrichment of DNA-response elements for nuclear receptors (ARE, GRE, and PGR) and the forkhead box family of transcription factors (FOXA1, a known pioneer factor for AR). The NE subtype lacked the ARE, GRE, and PGR motifs but was enriched with FOXA1 and neural-specific motifs including NEUROD1, ATOH1, OLIG2, and NANOG (Panel C).

Conclusion: Our results demonstrate significant heterogeneity in DSRCT phenotypes, where we observe both epithelial and NE lineages. Initially, we only detected the Epithelial/AR-positive and NE-positive lineages by gene expression, however, the chromatin accessibility data also supports this result. Next, using CytoTRACE, the NE subtype appeared to exist in a high-plasticity cell state unlike the Epithelial/AR-positive subtype, which directly parallels the relationship between Epithelial/AR and NE that is observed in PCa. The emergence of the NE subtype in PCa is generally in response to therapy. However, it is not known how NE emerges in DSRCT. The CytoTRACE analysis may suggest that NE may have de-differentiated from the more differentiated Epithelial/AR-positive subtype. Under active investigation, we are working to better understand the Epithelial/AR and NE in DSRCT dimorphic states. Given the presence of FOXA1 enriched motifs for both Epithelial/AR and NE, we have also begun to interrogate the FOXA1 cistrome to determine if it acts as a central regulator of DSRCT lineage specification.



(P 427) **UNDERSTANDING THE MOLECULAR DRIVERS THAT REGULATE EWING SARCOMA CELL FATE**

Danh D. Truong, PhD[1]; David McCall, MD[1]; Clement Nana Agyemang, MS[1]; Sandhya Krishnan, MS[1]; Alexander J. Lazar, MD, PhD[1]; Joseph A. Ludwig, IV, Ph.D.[2], (1) The University of Texas MD Anderson Cancer Center, Houston, TX, (2) Department of Sarcoma Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX

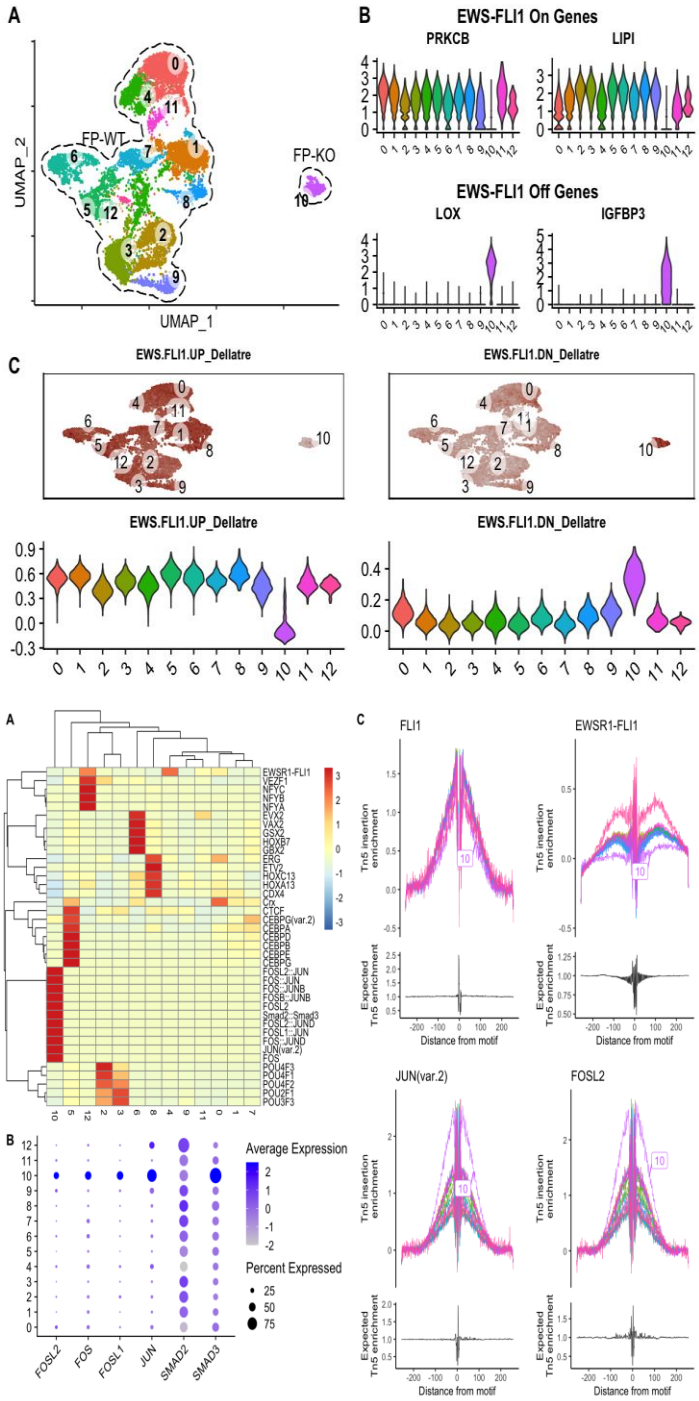
Objective: Like other translocation-positive sarcomas, Ewing sarcoma (ES) harbors a pathognomonic fusion protein (FP) capable of widespread epigenetic reprogramming that can lead cells to acquire a high-grade, undifferentiated phenotypic state. Though EWS-FLI1's action as an aberrant transcription factor with GGAA-microsatellite and ETS motif binding sites is well known, it remains to be determined precisely how its genome-wide stimulatory and repressive effects coalesce to block differentiation, alter cell plasticity, or promote a quasi-stem-like cell state that better resembles neuroectodermal cells or mesenchymal stem cells.
We first performed an unsupervised comparison of the ES transcriptome to other cancers available in the Cancer Cell Line Encyclopedia (CCLE) and existing tumor expression datasets to investigate this. Though traditionally classified as a 'bone cancer', ES cells clustered far from other sarcoma subtypes or malignancies. This unique transcriptional signature implied that the ES FP's effects are so broad sweeping as to invoke a ES-specific phenotypic state that strongly diverged from other known cell types. To better understand how this might occur, we employed a CRISPR knockout (CRISPR-KO) model of A673, targeting exon 4 of the N-terminus EWSR1 portion of the FP and generated a clonal pool (EWSR1 CRISPR KO). We used single-cell RNA and ATAC sequencing (scMultiome) to identify the transcriptional and chromatin changes after the FP knockout (FP KO).

Methods: To assess gene expression and chromatin accessibility, we performed Chromium Single Cell Multiome ATAC + Gene Expression on A673 WT and A673 EWSR1 CRISPR KO (EWSR1-KO) cells. Briefly, filtered gene barcoded matrices from Cell Ranger (10x Genomics) were processed by Seurat v4. Low-quality nuclei and additional outliers were removed. The data were log-normalized, and the top variable genes were selected for PCA dimensional reduction. ATAC-seq data were analyzed with the Signac package. Single cells were filtered for greater than 1,000 fragments, percent reads in peaks of at least 15%, nucleosome signals less than 10, and TSS enrichment score greater than 2. Data were normalized, followed by dimensional reduction with latent semantic indexing. We integrated the two data modalities using weighted neighbor clustering for integrative analysis of both RNA and ATAC data. chromVar detected motif enrichment per cell in sparse single-cell ATAC-seq data. Peak to gene annotations and transcription factor (TF)-gene regulatory network (GRN) were found by the Functional Inference of Gene Regulation (FigR) framework. We assessed differentiation by CytoTRACE, a computational tool designed to predict differentiation states.

Results: We identified eleven cell clusters of ES cell heterogeneity. Since the EWSR1-KO is a pooled population, we used known FP-regulated genes to delineate cells bearing a FP KO from wild-type (FP WT) cells. We observed robust expression of FP target genes (EWS-FLI1 On), PRKCB, and LIPI in ten clusters, suggesting regular FP function (FP WT). Conversely, FP repressed genes (EWS-FLI1 Off), LOX, and IGFBP3 were elevated in cluster 10, which

contained only EWSR1-KO cells. This suggests that the FP was successfully knocked out in cluster 10 (FP KO).
Next, we hypothesized that loss of the FP would induce a mesenchymal fate. To answer this, we used SingleR, which compares the gene expression profile of cancer cell lines to identify the closest cell type. The FP KO cluster did not resemble ES, but all other clusters identified closely to ES and related tumors. A granular identification with cell lines confirmed that all clusters except FP KO were identified as A673 cells. However, the FP KO resembled cell lines with a fibroblastic phenotype.
scMultiome enabled us to study the relationship between the FP target, repressed genes, and chromatin accessibility. We observed no significant difference between the FP KO and FP WT when investigating motifs for the FP or FLI1. However, the FP KO cells were enriched for motifs in the Fos/Jun family and Smad2/3, which have roles in EMT and bone and mesenchymal differentiation. The FP-KO cells significantly expressed the Fos/Jun family of proteins. Next, we identified peak-gene associations within a 500 kb window around the transcriptional start sites. We used HOMER motif discovery algorithm to search for motifs at these peaks. When we analyzed peaks linked to genes expressed in FP-WT, we found enrichment of the Fos/Jun protein family ($p < 1e-34$), Ets family, and GGAA-microsatellites. However, the FP-KO were strongly enriched for Fos/Jun protein family ($p < 1e-540$) with no change in Ets family motif.

Conclusion: Our data suggest that knocking out the FP in the A673 cell line significantly shifted the ES lineage. While the FP KO did not lead to cell death in the A673 cell line, our results suggest a novel gene regulatory network through the Fos/Jun family of proteins that may act to sustain an ES lineage that is not dependent on the FP. We observed that peaks linked to genes positively associated or regulated by the FP were enriched for Fos/Jun family, Ets family, and GGAA-microsatellites. This suggests a cooperative interaction between these TFs may drive the genes linked to the FP. Interestingly, upon losing FP, there was a significant enrichment of Fos/Jun family motifs but not Ets family or GGAA-microsatellites. This suggests that FP loss activates FP-repressed genes and enables chromatin remodeling to open previously inaccessible DNA binding sites.
Significant questions remain. It is unknown if opening Fos/Jun repressed sites with an active FP would still lead to a mesenchymal phenotype, and it remains to be determined whether our findings apply to other ES cell lines that poorly tolerate FP loss.



(P 428) **COMPREHENSIVE GENOMIC PROFILING OF CIC-DUX4 SARCOMA (CDS) CELL LINES AND MODELS**

Peter G. Hendrickson, MD,PhD[1]; MaKenna R. Browne, BS[2]; Kristianne M. Oristian, PhD[3]; Diana M. Cardona, MD, MBA[3]; Lixia Lou, BS[2]; Yan Ma, MS[3]; David Kirsch, MD, PhD[4], (1) Duke Univeristy, Durham, NC, (2) Duke University, Durham, NC, (3) Duke University, Duham, NC, (4) Princess Margaret Cancer Centre/University Health Network, Toronto, ON, Canada

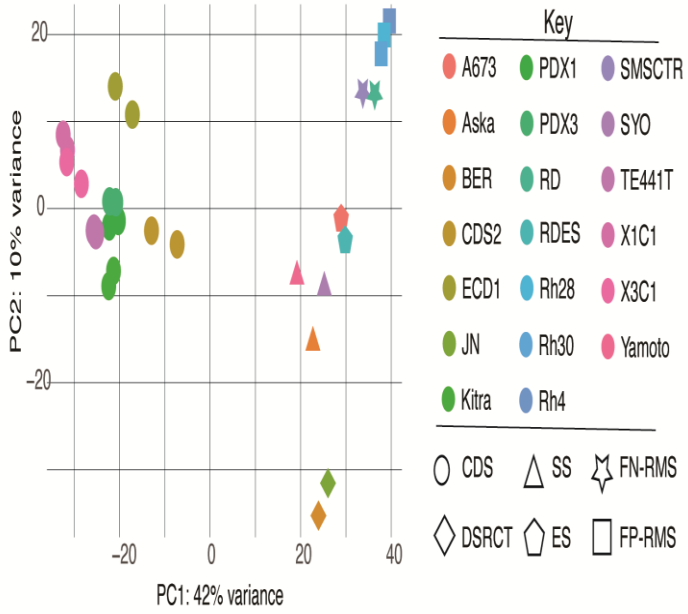
Objective: Ewing-like sarcomas constitute a rare and highly aggressive subtype of soft tissue sarcoma which histologically resemble Ewing but lack the Ewing-defining EWS-ETS gene fusion. In 2020, the W.H.O. began recognizing Ewing-like sarcomas as distinct disease entities which are now defined by the specific gene rearrangement or fusion these cancers possess. Although the incidence of these new small round cell sarcomas is not known, the most common appears to be driven by a translocation at t(4;19)(q35;q13) leading to a fusion between the DNA binding domain of capicua transcriptional repressor (CIC) with the transcriptional activation domain of double homeobox 4 (DUX4). CIC-DUX4 fusion-positive sarcomas (CDS) have an unfavorable clinical course and dismal 5-year overall survival attributable to its propensity for rapid and distant metastasis and poor response to conventional chemotherapies. For this reason, novel therapeutic strategies and treatments are desperately needed.

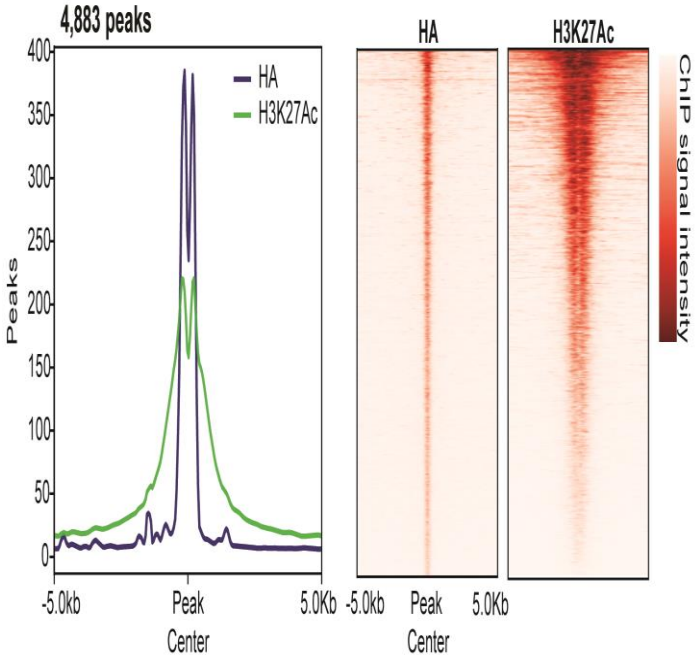
Methods: To elucidate a mechanism of disease initiation/maintenance and identify CDS-specific vulnerabilities, we comprehensively profiled human and mouse CDS cell lines. Review of the literature and public databases identified eight cell lines harboring a CIC-DUX4 gene fusion including four primary cell lines (ECD1, NCC-CDS2, Kitra-SRS, and TE441.T) and four mouse xenograft-derived cell lines (NCC-CDS-X1C1, NCC-CDS-X3C1, PDX-CDS1, PDX-CDS3). Additionally, two primary cell lines derived from spontaneous tumors in a genetically engineered mouse model of HA-CIC-DUX4 sarcoma were included (CDS-001, CDS-108). RNA-sequencing, Assay for Transposase-Accessible Chromatin (ATAC)-sequencing, and Chromatin Immunoprecipitation (ChIP)-sequencing were performed. Immunoprecipitation reactions were prepared using antibodies against the DUX4 CTD (abcam: ab124699), HA-epitope tag (abcam: ab9110), and H3K27ac (Active Motif 39134). 150bp paired-end libraires were prepared and sequenced on an Illumina HiSeq platform. Raw reads were aligned using STAR. Differential gene expression was called using DESeq2 relative to other small round cell sarcoma cell lines including Ewing sarcoma (ES) [RD-ES, A-673] desmoplastic small round cell sarcoma (DSRCT) [BER-DSRCT, JN-DSRCT], synovial sarcoma (SS) [Yamoto-SS, Aska-SS, SYO1], fusion-negative rhabdomyosarcoma (FN-RMS) [RD, SMS-CTR], and fusion-positive rhabdomyosarcoma (FP-RMS) [Rh4, Rh28, Rh30] all of which were sequenced in parallel. ATAC-seq peaks and ChiP-seq peaks were called using macs3 and then integrated to determine the core regulatory circuity using the ROSE and Coltron computational pipelines.

Results: Principal Component Analysis (PCA) separated the 20 small round cell sarcoma cell lines into two primary clusters based on global RNA expression profiles. Cluster 1 was composed of the eight human CDS cell lines and cluster 2 was composed of all other small round cell sarcomas (‘other’) which further segregated according to type along principal component 2 (figure 1). Differential expression analysis comparing CDS to ‘other’ revealed 2,054 upregulated genes (log2FC> 1, padj < 0.01) and 2,760 downregulated genes (log2FC< 1, padj < 0.01). CIC-DUX4 ChIP-seq using an anti-DUX4 antibody and anti-HA antibody identified 1,386 and 1,043 direct downstream targets (+/- 3kb of TSS) in human and mouse respectively. 72 direct downstream targets were conserved between the two species including: ETV1, ETV4, ETV5, FOS, SHC3, SCH4, DUSP4, DUSP6, and VGF. Regions bound by CIC-DUX4 were also enriched for H3K27ac and open chromatin as evaluated by ATAC-seq consistent with the notion that CIC-DUX4 is behaving as a neomorphic transcriptional activator in CDS (figure 2). Interestingly, CIC-DUX4 bound regions in human and mouse cell lines were also strongly enriched for a de novo motif matching the consensus ‘ETS’ transcription factor binding site

[GGAA]. Further analysis of Super-Enhancer associated transcription factor binding suggests that ETV1/4/5 are key mediators of the core regulatory circuitry (CRC) in CDS.

Conclusion: Identifying therapeutic vulnerabilities and novel treatment strategies necessitates a firm understanding of disease mechanisms. Because of its rare incidence, human tissues are not readily available however numerous cell lines containing the CIC-DUX4 gene fusion have been established; in some cases, unknowingly. Here we obtained these cell lines and used Next Generation Sequencing based approaches to comprehensively profile the transcriptional and chromatin landscape of CDS in human and mouse. These datasets suggest that CDS is distinct from other small round cell sarcomas including ES, DSRCT, SS, and RMS. This is underscored by its unique transcriptional profile and enrichment at gene promoters. We also found that CIC-DUX4 occupancy strongly correlates with H23K27ac, open chromatin, and transcriptional upregulation in human cell lines which is recapitulated at notable targets in tumors derived from mice genetically engineered to express the CIC-DUX4 fusion gene. Taken together, these data strongly support the notion that CIC-DUX4 represents a neomorphic transcriptional activator. It also points to an unexpected co-operation between CIC-DUX4 and the Pea3-subfamily of ETS transcription factors in regulating the CDS transcriptional program.





(P 429) SARCOMA FUSION GENE ANALYSIS YIELDS PROMISING HLA ANTIGEN FIT IN EWING SARCOMA AND DESMOPLASTIC SMALL ROUND CELL TUMOR

Peter M. Anderson, MD, PhD; Zheng Jin Tu, PhD; Vladimir Makarov, MD, MS; Gyanu Parajuli, PhD; Suheyla Hasgur, DVM, PhD; Jeffery A. Knauf, PhD; Timothy A. Chan, N/A, MD PhD, Cleveland Clinic, Cleveland, OH

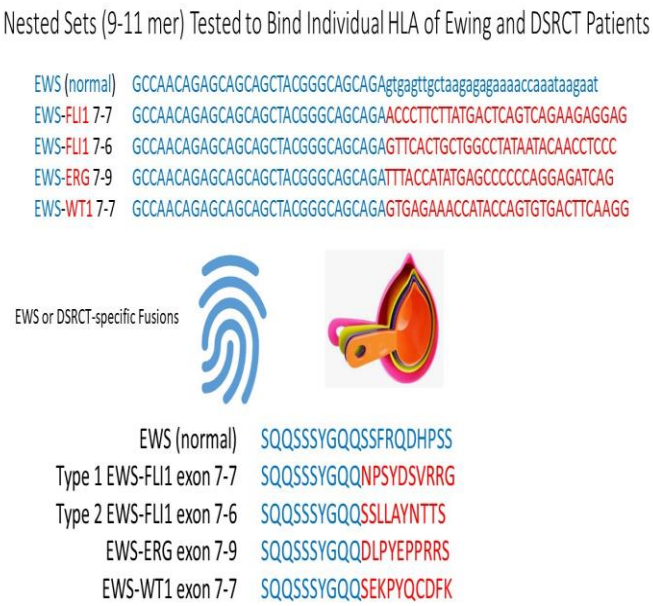
Objective: EWS gene fusions are cancer-specific events in both Ewing sarcoma and Desmoplastic Small round Cell Tumor (DSRCT). We sought to determine whether or not EWS sarcoma gene fusions code for potentially immunogenic peptides that span the fusion breakpoint by analyzing patterns of immunogenicity (common or uncommon) in the context of a Ewing sarcoma or DSRCT patient's individual major histocompatibility (MHC) class I HLA-A, -B, and C type. Our overall objective was to identify potential candidate antigens to be coded for in EWS-fusion adenovirus and mRNA vaccines suitable for the majority of Ewing sarcoma and DSRCT patients.

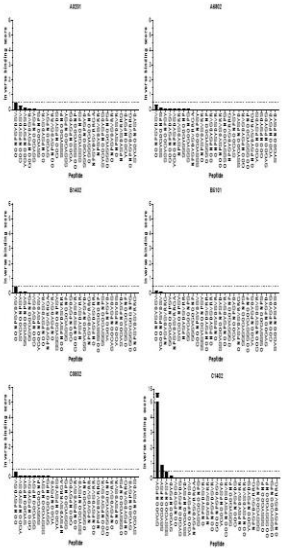
Methods: EWS breakpoint and HLA data was obtained from Cleveland Clinic Molecular Pathology NGS laboratory and Caris Life Sciences (Irving, TX). In-frame analysis was performed to determine fusion DNA, RNA, and peptide sequences spanning the EWS breakpoint for each patient. Then, we looked for similarities and differences of peptides encoded by EWS-FLI1, EWS-ERG, EWS-FUS, and EWS-WT1 gene fusions. We then analyzed nested sets (27/HLA allele) of fusion peptides (9-11 mer) spanning the EWS-FLI1, EWS-ERG, EWS-FUS, and EWS-WT1 breakpoints in the context of individual predicted HLA binding of

HLA-A, -B, and -C alleles from >45 patients with Ewing sarcoma and DSRCT. Patterns of excellent, good, and poor EWS-fusion peptide antigen fit were then characterized.

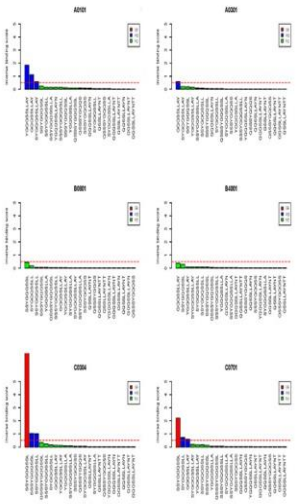
Results: : Almost every patient had at least one predicted EWS-fusion peptide that was predicted to be presented by MHC. Interestingly, after analysis of >45 patients, common patterns emerged for each EWS- fusion HLA-fit. EWS-FLI1 type 1 and EWS type 2 had 5 and 4 good fits, respectively. Less common EWS-FLI1 fusions also had good fits. EWS-ERG had 4 fusion antigens that appeared to be presented; EWS-WT1 had 3 breakpoint-spanning peptides that were predicted to bind MHC. Although 9-mer EWS fusion antigens were the most common, MHC also was predicted to bind some, 10- and 11 -mer peptides too. A total of 22 “best” candidate EWS-fusion peptides could be potential targets that would be relevant to> 80% of Ewing sarcoma and DSRCT patients. Validation using experimental means may be used to examine these candidates in more detail.

Conclusion: : A surprisingly small, but potentially useful subset of EWS-FLI1, EWS-ERG, EWS-FUS, and EWS-WT1 fusion peptides are predicted to be highly immunogenic in the context of individual class I HLA binding. This finding has guided the design of EWS vaccine constructs that should be relevant to the majority of high-risk Ewing sarcoma and all DSRCT patients. If our method is applied to other fusion-associated sarcomas, it will also likely yield potential vaccine information for common and uncommon sarcoma fusions including other round cell sarcomas such as PAX7-FOXO1 and PAX3-FOXO1 alveolar rhabdomyosarcoma, SS18-SSX synovial sarcoma, BCOR-CCNB3 sarcoma, and CIC-DUX sarcoma.

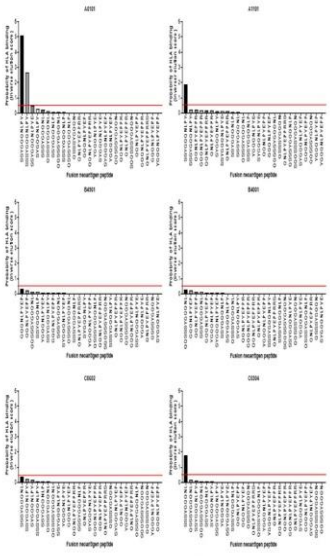




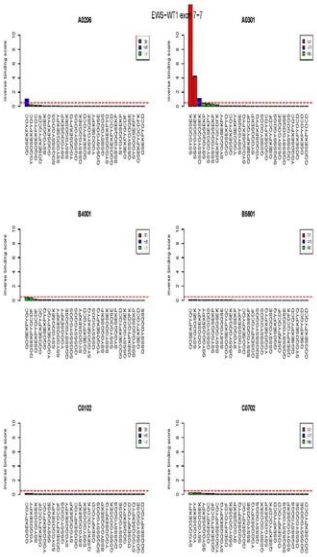
Type 1 SYGQQNPSY and HLA C1402 (5-4 9-mer) are best of 162 EWS-FLI Type 1 HLA binding combinations



Type 2 SSYGQQSSLL(6-4 10-mer) is an excellent binder for both HLA C0304 and HLA C0701



EWS-ERG fusion SSYGQQNLPY (6-4 10–mer), but also SSSYGQQNLPY (7-4 11-mer) bind HLA A0101, HLA A1101 and HLA A0304; the other 158 HLA EWS-ERG fusion combinations have less binding.



EWS-WT1 fusion SSSYGQQSEK (7-3 10-mer) and SSSYGQQSEK (8-3 11-mer) bind HLA A0301. The other 160 HLA –EWS WT1 combinations are less avid.

Excellent HLA Binders for EWS fusions

EWS-Fusion Gene	Immunogenic DNA/mRNA/peptides (N)
EWS-FLI1	14
EWS-FEV	1
EWS-ERG	4
EWS-WT1	3
Total	22

(P 430) UNIQUE HISTOLOGIC MORPHOLOGY OF EWING SARCOMA CELLS THAT HARBOR TP53 MUTATIONS

Christopher Kuo, MD[1]; Anahit Nargizyan, MPH[2]; Yueh-Yun Chi, PhD[2]; Rachana Shah, MD[2]; Fariba Navid, MD[3]; G. Esteban Fernandez, PhD[2]; James Amatruda, MD, PhD[2]; Leo Mascarenhas, MD, MS[4]; Mikako Warren, MD[2], (1) Children's Hospital Los Angeles, Glendale, CA, (2) Children's Hospital Los Angeles, Los Angeles, CA, (3) Children's Hospital Los Angeles, Los Angeles, CA, (4) Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA

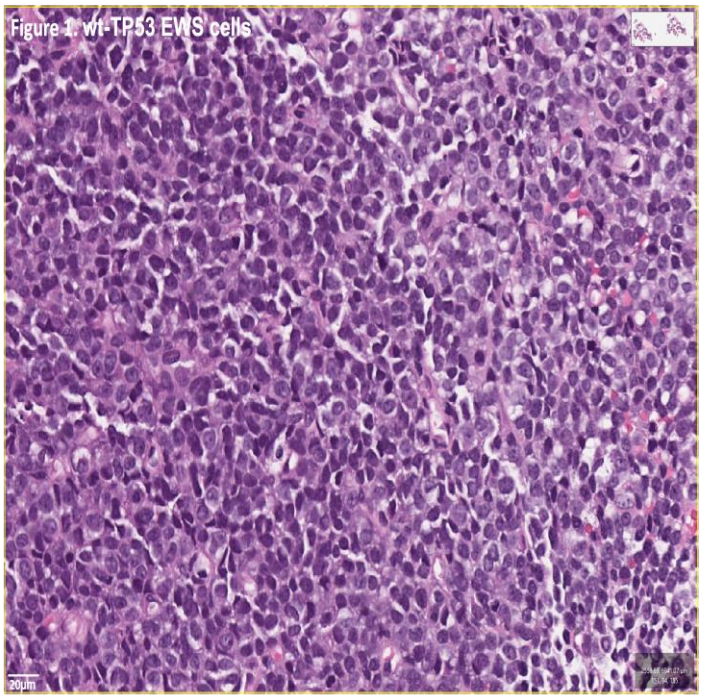
Objective: Ewing sarcoma (EWS) often harbors TP53 mutations in 4% to 20% of cases. Patients with TP53 mutations are reported to have a poor prognosis, with more invasive and less chemoresponsive tumors. Immunohistochemistry (IHC) staining for TP53 is not routinely performed at diagnosis and while molecular testing reliably detects TP53 mutations, results are not immediately available. There have been no reports on the morphology of EWS cells that harbor TP53 mutation. The objective of this retrospective study is to describe and compare the histologic appearance of TP53 mutated (mut) EWS cells from patients' tumor biopsy samples to tumors that do not harbor TP53 mutations.

Methods: A pilot single-institution retrospective medical record and histopathologic review of a small cohort of patients with EWS who underwent tumor biopsy from 2017-2023. Hematoxylin and eosin (H&E) stained slides were reviewed by an expert pathologist to select non-necrotic,

tumor dense region of interest (ROI). Slides were scanned and reviewed independently by a researcher who was blinded to EWS TP53 mutation status. QuPath software is then used to measure nuclear area (NA) (µm^2) and nuclear optical density range (NODR) for each cell. The median and interquartile range (IQR) were calculated to summarize the measurements for NA and NODR. A Mann-Whitney test was used to evaluate the differences by TP53 mutation status. The study was approved by the Institutional Review Board prior to data collection (CCI-06-00198).

Results: Twelve patients with EWS were selected, 6 (50%) were metastatic at diagnosis; Ten (83%) had EWS::FLI fusion and 2 (17%) had EWS::ERG. A total of 14 tumor slides were assessed, with 9 (64%) obtained at diagnosis; 4 (29%) at 1st recurrence; 1 (7%) at 2nd recurrence. Eight (57%) were primary tumor biopsies; 1 metastatic and 5 relapse/recurrent biopsies. Half of the tumors (n=7) harbored TP53 mutations (mut-TP53). All TP53-wild-type (wt-TP53) EWS cells showed monotonous oval nuclei with fine chromatin pattern and inconspicuous nucleoli (Figure 1). In contrast, all mut-TP53 EWS cells demonstrated enlarged oval-to-round nuclei with a coarse chromatin pattern with 1-2 prominent nucleoli (Figure 2). Summary statistics of NA were comparable between wt-TP53 and mut-TP53 cells with a median NA median of 26.28 µm^2 (21.99-30.58 µm^2) and 24.77 µm^2 (18.45-29.06 µm^2), respectively; p=0.52, and a median NA IQR of 19.46 µm^2 (12.64-27.04 µm^2) vs. 17.06 µm^2 (14.15-24.01 µm^2), respectively; p=0.7 (Figure 3A/B). Summary statistics of NODR were comparable between wt-TP53 and mut-TP53 cells with a median of median NODR of 1.03 (0.83-1.29) vs. 0.94 (0.67-1.36), respectively; p=0.34, and a median NODR IQR of 0.44 (0.31-0.52) vs. 0.48 (0.33-1.00), respectively; p=0.57 (Figure 3C/D).

Conclusion: In this limited sample study, there was no significant difference in NA and NODR distributions of mut-TP53 compared to wt-TP53 EWS (Figure 4). However, EWS cells with mut-TP53 have a unique appearance on H&E morphology to those with wt-TP53, losing their classic monotonous small round blue cell appearance. As outcomes for patients with localized EWS continue to improve, the ability to predict TP53 mutational status at diagnosis will be an important tool to risk stratify patients, pre-treatment. We are expanding this pilot study to increase statistical power in assessing the prognosis of nuclear morphologic characteristics, NA and NODR.


Figure 1. wt-TP53 EWS cells
20µm

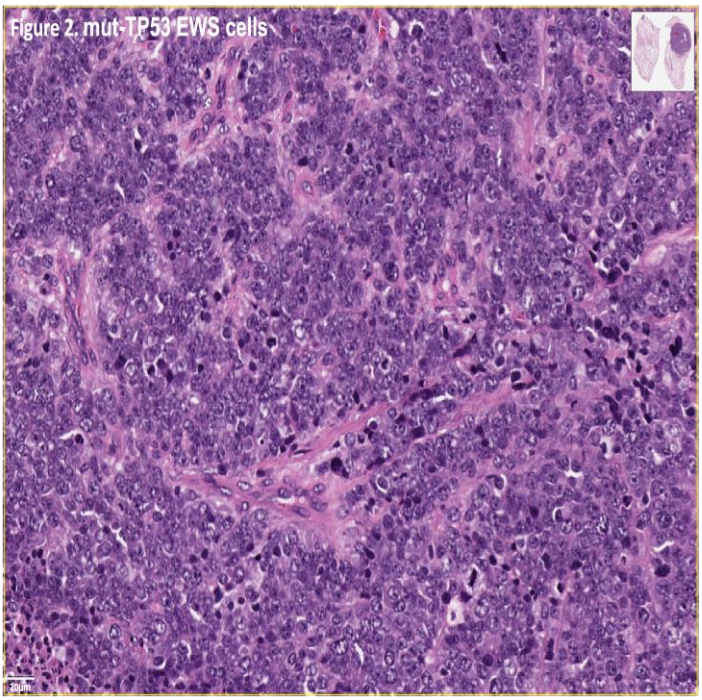

Figure 2. mut-TP53 EWS cells
20µm

Figure 3. Median and Interquartile range (IQR) by TP53 mutation status

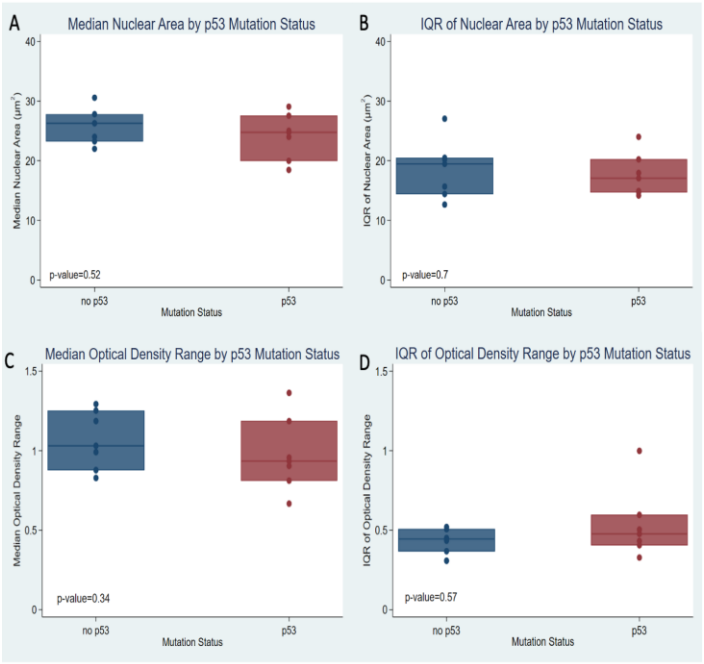
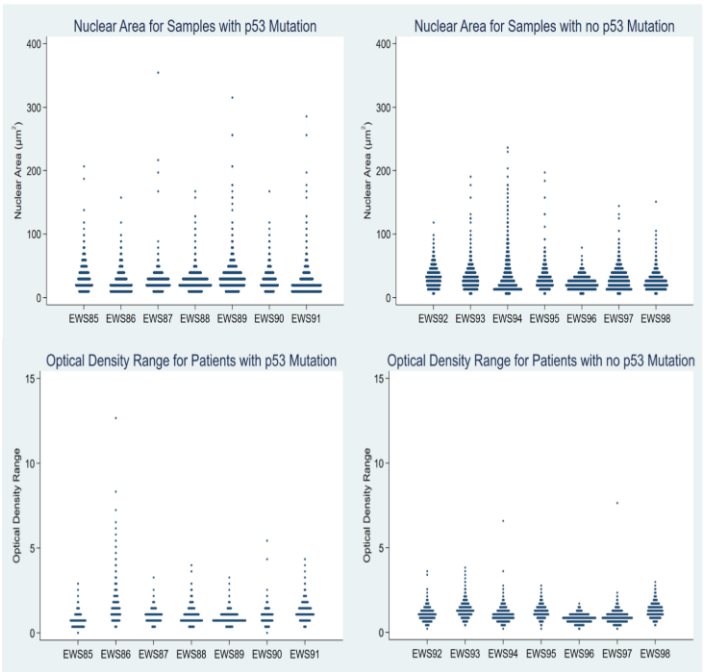


Figure 4. Nuclear Area and Optical Density Range between patients with wt-TP53 or mut-TP53



(P 431) TREATMENT STRATEGY AND PROGNOSTIC FACTORS IN EXTRASKELETAL EWING SARCOMA: AN INSTITUTIONAL RETROSPECTIVE ANALYSIS

Giulia Apollonio, MD[1]; Caterina Zanella, MD[2]; Rossella Bertulli, MD[3]; Paolo Giovanni G. Casali, MD[4]; Paola Collini, MD[5]; Antonina Parafioriti, MD[6]; Elisabetta Armiraglio, MD[6]; Alessandro Gronchi, MD, FSSO[7]; Alessandro Luzzati, MD[8]; Primo Andrea Daolio, MD[6]; Ugo Pastorino, MD[9]; Claudia Sangalli, MD[10]; Albina Allajbej, MD[2]; Dario Callegaro, MD[11]; Salvatore Provenzano, MD[3], (1) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (2) Fondazione IRCCS Istituto Nazionale dei Tumori, milan, Lombardia, Italy, (3) Mesenchymal and rare cancer Medical Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (4) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (5) Department of Advanced Diagnostics , Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (6) ASST Gaetano Pini - CTO, milan, Lombardia, Italy, (7) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (8) IRCCS Galeazzi Orthopedic Institute, milan, Lombardia, Italy, (9) Fondazione IRCCS Istituto Nazionanale dei Tumori, Milano, Lombardia, Italy, (10) Radiation Oncology Department, Fondazione IRCCS Istituto Nazionale Tumori, Milano, Lombardia, Italy, (11) Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy

Objective: Extraskeletal Ewing sarcoma (EES) is an aggressive and rare small round cell neoplasm, more commonly marked by EWSR1-2::FLI1 translocation. Whether it is associated with a different prognosis comparing to its skeletal, more common, counterpart is still to be defined. The aim of our study is to analyze treatment strategy, prognostic factors, and survival rates in patients (pts) with EES.

Methods: We conducted a retrospective analysis searching for all consecutive adult pts treated with chemotherapy (CT) for EES at the Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy. Data on pts characteristics were retrieved and analyzed. A univariate survival analysis with log-rank test was made, and overall survival (OS) and event-free survival (EFS) were estimated using the Kaplan–Meier method.

Results: Between 1983 and 2022, we identified 122 adult pts (median age = 34) with diagnosis of EES; herein, we present the data of the 56 pts with a diagnosis confirmed by the identification of EWS/ETS family transcript. Patient characteristics, treatment and responses are summarized in Table 1 and 2. At a median follow-up of 73.5 mos, the 5-year OS was 73.5% and the 10-year OS was 64%. The 5-year OS was 84% in pts with localized disease and 25% in metastatic pts. The univariate analysis was performed on pts with localized disease. A trend to worse OS was observed in pts with tumor size ≥8 cm at the diagnosis (p= 0.12). Complete R0 resection was significantly associated with better OS and EFS compared to R1 or R2 (p< 0.0001 and p= 0.03 respectively). No difference in outcome was seen between different local treatment modalities (surgery alone, definitive radiotherapy or surgery plus radiotherapy). Median EFS rate was significantly less favorable in pts with tumors ≥8 cm (p=0.02). Pts achieving CR/PR/SD according to RECIST following induction CT had respectively 100/80/40% 5-year EFS (p=0.01). No difference in OS was seen between pts with CR and PR. Complete pathological response (pCR) was not statistically significantly associated with a better outcome compared to no pCR.

Conclusion: EES is generally underrepresented in clinical trials and/or is analyzed together with its bone counterpart. In localized disease, tumor size, adequate surgical resection and

radiological response to induction CT were significantly related to outcome. We found a strong correlation between clinical response and the outcome, apparently higher than for pathologic response. The choice of local treatment was less critical for outcome than expected, confirming that also in EES a multidisciplinary treatment may be effective in selected cases also without surgery.

Table 1. Patients' characteristics

	Number	Percentage (%)	Range
Sex			
Female	24	43	
Male	32	57	
Age			16 - 74
<40	37	66	
≥40	19	34	
Stage			
Localized	45	81	
Metastatic	11	19	
Primary Tumor Site			
Head and Neck	5	9	
Extremities	12	21	
Chest	10	18	
Abdomen/retroperitoneum	19	34	
Back	10	18	
Tumor size			1,2 - 30
≥8 cm	23	41	
<8 cm	25	45	
ND	8	14	
Metastatic sites			
Lung	7	12	
Liver	3	5	
Bone	7	12	
Lymphnodes	1	2	
Others (soft tissues)	3	5	

Table 2. Patients' treatments and responses

	Number	Percentage (%)	Range
Systemic Treatment			
Median number cycles	8,5		3 - 12
Peri-operatory	26	46	
Pre-operatory	7	12	
Post-operatory	10	18	
First line	9	16	
Surgery			
Yes	43	77	
No	12	21	
ND	1	2	
Radiotherapy			
Pre-operatory	4	7	
Post-operatory	22	39	
Definitive	3	5	
Palliative	3	5	
None	20	36	
Surgical margins			
R0	34	61	
R1	2	3	
R2	2	3	
ND	18	32	
Radiological Response (RECIST)			
SD	5	9	
PR	34	61	
CR	10	18	
PD	4	7	

(P 432) LONG-LASTING DISEASE CONTROL WITH TRABECTEDIN IN A CASE OF METASTATIC EWSR1::PATZ1 SARCOMA

Silvia Brotea-Mosoiu, MD[1]; Giulia Apollonio, MD[2]; Rossella Bertulli, MD[3]; Claudia Sangalli, MD[4]; Paola Collini, MD[5]; Ugo Pastorino, MD[6]; Salvatore Provenzano, MD[3], (1) Oncology Institute of Bucharest, Bucharest, Bucuresti, Romania, (2) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Lombardia, Italy, (3) Mesenchymal and rare cancer Medical Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (4) Radiation Oncology Department, Fondazione IRCCS Istituto Nazionale Tumori, Milano, Lombardia, Italy, (5) Department of Advanced Diagnostics , Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (6) Fondazione IRCCS Istituto Nazionanale dei Tumori, Milano, Lombardia, Italy

Objective: EWSR1::PATZ1 translocation associated neoplasms include glioneuronal tumors, cases of kidney neoplasms and head and neck, chest and abdominal wall spindle cell or small round cell sarcomas. Their molecular and clinical features are not fully known. Treatment is not standardized, and the multidisciplinary approach in sarcoma cases is derived from protocols commonly used in Ewing or other soft-tissue sarcomas. We describe a case of a long-lasting metastatic disease controlled by trabectedin in a young female patient with a small round cell EWSR1::PATZ1 sarcoma of the chest wall.

Methods: In June 2015, a 28 years old female was diagnosed with a mesenchymal small round cell neoplasm of the chest wall. Immunohistochemistry: CD99-, vimentin+, desmin+, WT19+, WT180-. Fluorescence In-Situ Hybridization revealed EWSR1 gene translocation. No partner was found at that time. The patient underwent 5 courses of pre-operative chemotherapy with vincristine/doxorubicin/ifosfamide, and one cycle of etoposide/ifosfamide with an initial partial response, which was lost after the fifth cycle. En-bloc complete resection with prosthetic reconstruction, adjuvant chemotherapy with etoposide and ifosfamide for 3 more courses and external-beam radiotherapy (60Gy) followed. After 4 years, the disease relapsed with multiple pleural/chest wall ipsilateral metastases. The relapse was confirmed by biopsy, which revealed the presence of a EWSR1::PATZ1 translocation. The patient underwent complete macroscopic resection of all metastases and remained in follow up for the following 2 years, when another multinodular recurrence was clinically confirmed. Lacking evidence on the management of this histotype, the patient started trabectedin in March 2021.

Results: To date, after 33 cycles over two years, the patient remains on treatment and formally stable according to RECIST. Overall, the treatment is well tolerated and side effects were, as expected, G2 nausea, asthenia, and neutropenia, the latter controlled with q21 to q28 interval prolongation and one-level dose reduction.

Conclusion: Although a formal RECIST response was not observed, we provide evidence on the possible efficacy of trabectedin in a very rare sarcoma histotype, lacking protocols on the proper management. To our knowledge, this is the first case report on trabectedin use in EWSR1::PATZ1 small round cell sarcoma, in the literature. Given its rarity, large, prospective trials on this histotype remain very unlikely to be run. Real-world data collection is encouraged.

(P 433) A 20-YEAR MONOCENTER EXPERIENCE ON CLINICAL CHARACTERISTICS AND OUTCOME OF EXTRASKELETAL EWING SARCOMA.

Alexia Francesca Bertuzzi, MD[1]; Vittorio Quagliuolo, MD[2]; Piergiuseppe Colombo, MD[3]; Salvatore Lorenzo Renne, MD[4]; Nicolo Gennaro, MD[5]; Alice Laffi, MD[4]; Maria Susanna Grimaudo, MD[6]; Laura Giordano, MS[1]; Umberto Cariboni, MD[1]; Pierina Navarria, MD[1]; Andrea Marrari, N/A, MD[7]; Federico D'Orazio, MD[1]; Licia Siracusano, MD[1]; Stefania Bramanti, MD[1]; Armando Santoro, Prof[1], (1) Humanitas Research Hospital, Rozzano, Lombardia, Italy, (2) Humanitas, Research Hospital, Milan, Italy, Rozzano, Lombardia, Italy, (3) IRCCS Humanitas Research Hospital, Milano, Lombardia, Italy, (4) IRCCS Humanitas Research Hospital, Rozzano, Milan, Lombardia, Italy, (5) Northwestern University Feinberg School of Medicine, Chicago, IL, (6) Humanitas Research Hospital, Milano, Lombardia, Italy, (7) Istituto Ortopedico Rizzoli, Bologna, Emilia-Romagna, Italy

Objective: Extraskeletal Ewing sarcoma (EES) represents a rare entity, historically described as the soft tissue counterpart of the classical bone Ewing Sarcoma (ES), including Askin tumors and peripheral primitive neuroectodermic tumor (pPNET). Morphologically, they belong to the small round blue cells sarcoma family.
Compared to the ES, EES is more common in adults and young adult (AYA) patients (pts) rather than paediatric population. Besides incidence and clinical presentation, the outcome and the molecular/biological characteristics markedly differ among the two ES groups. So far, the available data on this topic originate from multicenter, paediatric ES studies, and the treatments have replayed the therapeutic approaches generally adopted for the ES.
Furthermore, for both EES and ES, the molecular pattern prognostic impact is still debated. This retrospective analysis aimed to describe the clinical features, the peculiar behaviour and the outcomes of a monocenter EES population. Beside the classical fusion-transcript, new entities have been identified (CIC-DUX and BCOR), which requires a further personalized approach.

Methods: We collected and analysed the data (demographic, clinical, pathological and radiologic data) of all subsequent EES treated in a tertiary cancer center between 1997 to 2023. Based on the clinical presentation at diagnosis (extremities vs axial site, tumour volume and stage) and according to the previous published bone ES data, we identified three risk categories: 1) standard risk (localized disease, susceptible to radical surgery), 2) high risk (locally advanced, usually axial site and over than 5 cm, and pulmonary metastasis and 3)very high risk (presence of extrapulmonary metastatic patients). Each available pathological specimen has been review by a sarcoma-dedicated team of pathologists, with the molecular assessment still ongoing for the final data. The retrospective analysis was approved by our Etic Committee.

Results: We identified 76 pts, 47 males (62%), with a median age at diagnosis of 26 (15-70). 3 (4%) were categorized into standard risk, 58 (76%) high risk and 15 (20%) very high risk; 14 pts had an Askin tumour (18%) and they all were categorized as high risk sarcoma.
27 pts presented with metastatic disease at diagnosis. Among the 41 pts with available molecular data, 35/41 (85%) presented an ets-related fusion transcript. At the staging, the bone marrow biopsy was performed on 55 patients (72%), with only 3 pts positive. 14/49 localized/locally advanced disease pts had surgery upfront, due to the clinical presentation. The remaining had chemotherapy upfront as indicated in ES therapeutic management. 40 (53%) received high dose chemotherapy after standard treatment based on the risk category. In terms of local treatment, 11 pts had radical surgery (upfront or after induction chemotherapy), 10

received definitive radiotherapy, 24 pts have been treated with both local treatment (49%) due to soft tissue involvement. The whole 5-years event-free survival (PFS) and overall survival (OS) were 35% and 41%, respectively.
We stratified the outcomes pts based on stage (metastatic diseases vs localized/locally advanced disease) and on risk category (very high risk vs standard/High risk), suggesting a statistical difference between the groups. Furthermore, with this analysis (Table 1) we also demonstrated the impact of non pulmonary metastases on EES pts prognosis. An updated analysis will be provided at the time of the meeting.

Conclusion: According to our knowledge, this analysis is the largest monocentric study on EES and represents a useful instrument to better understand this rare entity, affecting both adolescent & young adult (AYA) and adult patients. We showed the prognostic impact of non-pulmonary metastatic disease (classified as the very high risk category)on EES pts, suggesting an comparable survival rate for patients with pulmonary metastatic disease and those with locally-advanced disease. Further prospective studies are needed to explore and demonstrate this hypothesis.

	5 ys PFS	Logrank p value	5 ys OS	Logrank p value
Very high risk pts	7%	0.002	12%	0.008
Standard + high risk pts	42%		47%	
Metastatic pts	8%	<0.001	11%	<0.001
Localized/locally advanced pts	49%		56%	

Table 1: 5-years event-free survival (PFS) and overall survival (OS) according to each category of pts.

(P 434) MECHANISMS OF CHEMORESISTANCE IN CIC-DUX4 SARCOMA AS DETERMINED BY IN VIVO FUNCTIONAL GENOMICS

Masoumeh Aghababazadeh, N/A, PhD Student[1]; Ana Castillo Orozco, PhD Candidate[2]; Marjan Khatami, PhD student[2]; Geoffroy Danieau, PhD[2]; Niusha Khazaei, PhD Candidate[2]; Wajih Jawhar, PhD Student[2]; Swneke Donovan Bailey, PhD[1]; Livia Garzia, PhD[2], (1) McGill University, Montreal, PQ, Canada, (2) Research Institute of the McGill University Health Center, Montreal, PQ, Canada

Objective: Sarcomas are malignant tumors of mesenchymal origin. Although considered rare, they affect many children and young adults accounting for 20% of pediatric malignant

neoplasms. A recently described fusion-driven sarcoma, Capicua (CIC) rearranged sarcoma, is similar in appearance to Ewing family tumors (EFTs) but their biology and clinical course are different. CIC-DUX4 is the most common fusion in this type of sarcoma. Because of their previous classification as EFTs and their rare incidence, studies on these tumors have been sparse and the molecular pathways that cooperate with CIC-DUX4 in tumorigenesis, therapy failure, and metastatic dissemination are poorly described.

Methods: To understand which genes and pathways cooperate with CIC-DUX4, we have applied a high-throughput functional genomics screening, based on random insertional mutagenesis with transposons, in two cell lines with CIC-DUX4 sarcoma. In our system, engineered transposons are delivered to the target cells by lentiviral transduction and mobilized to create gain-of-function and loss-of-function mutations by the action of an inducible transposase. Engineered CIC-DUX4 tumor cells are implanted in immunocompromised mice, subcutaneously and orthotopically. Tumor progression and metastasis are monitored using bioluminescence imaging, primary tumors, relapses, and metastasis will be harvested, followed by next-generation sequencing molecular profiling. To discover drivers of chemo resistance in this context, we developed a fractionated high-dose chemotherapy protocol to use on engineered CIC-DUX4 implanted mice. This regimen is based on the same genotoxic chemotherapy that is currently in use for CIC-DUX4 patients: VDC-IE (vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide).

Results: Two different breakpoints for the CIC-DUX4 fusions were detected in the two cell lines and the fusion transcripts have been confirmed. The cells were not metastatic or low metastatic at baseline, and three cycles of chemotherapy treatment significantly improved the overall survival of the mice. Our human cell based, functional genomics model with transposons resulted in CIC-DUX4 tumors with a significantly higher proclivity to metastasize. For instance, the metastatic rate went from 10 to 60% metastases to the lungs or liver in the mice that received three cycles of chemotherapy. Transcriptomic profiling of the resistant tumors showed enrichment in pathways regulating pluripotency such as Wnt, TGF-b, and Notch. Increased representation of cancer stem cell markers such as CD44, ALDH1A1, and Sox2 was a significant feature of tumors chemotherapy-resistant tumors.

Conclusion: Functional genomics can be used in vivo in rare cancers to model cancer evolution during therapy. A somatic mutagenesis-based approach to model recurrent CIC-DUX4 tumors showed that genetic driver events support the evolution of CIC-DUX4 tumors to a more aggressive phenotype under chemotherapy-induced selective pressure. The genetic basis of this phenotype is being mined to identify actionable candidates to prevent or treat CIC-DUX4 sarcoma progression.

(P 435) **ONCOGENIC FUSION YWHAE::NUTM2 DISRUPT EP300/BCOR INTERACTION**

Mehdi Brahmi, MD PhD[1]; Adeline Duc[1]; Helene Vanacker, N/A, MD[1]; Virginie Perrin, n/a[1]; Daniel Pissaloux, PhD[1]; Armelle Dufresne, MD PhD[2]; Marie Karanian, MD PhD[1]; Waisse Waissi, MD PhD[1]; Nadège Corradini, MD[2]; Jean-Yves Blay, MD, PhD[3]; Franck Tirode, PhD[1], (1) CENTRE LEON BERARD AND CANCER RESEARCH CENTER OF LYON (INSERM U1052-CNRS5286), LYON, Rhone-Alpes, France, (2) CENTRE LEON BERARD, LYON, Rhone-Alpes, France, (3) Centre Léon-Bérard, Lyon, Rhone-Alpes, France

Objective: Several groups of primitive round cell sarcomas harbor YWHAE::NUTM2 oncogenic fusion, resulting in BCOR overexpression. However, YWHAE::NUTM2 mechanisms of oncogenesis and roles in BCOR overexpression have not yet been well described. With the aim to provide a comprehensive description of YWHAE::NUTM2 oncogenic mechanism, we investigated by RNA-sequencing a retrospective series of tumors and performed mass spectrometry across transfected cell lines.

Methods: This study was performed as a Centre Léon Bérard (Lyon, France) monocentric retrospective translational research program on tumor samples from an investigational cohort of 11 YWHAE::NUTM2 sarcomas: 8 endometrial stromal sarcomas and three undifferentiated round-cell sarcoma (two neonatal soft tissue/visceral sarcoma and one adult bone sarcoma). Whole exome RNA sequencing was performed with FFPE material in all cases using TruSeq RNA Access Library Prep Kit (Illumina®, San Diego, USA). After the YWHAE::NUTM2 fusion was cloned and sequenced, we established the expressing FLAG-tagged plasmid pIRES-YWHAE::NUTM2 and transfected it into the human cervical cancer cell line HeLa, using Neon electroporation. Then, we performed protein extraction and FLAG immunoprecipitation followed by mass spectrometry in order to identify and characterize YWHAE::NUTM2 oncoprotein interactions.

Results: RNAseq analysis revealed YWHAE fusions in all 11 patients including 8 involving NUTM2E, two invoving NUTM2B and one involving NUTM2A. Furthermore, it showed significant higher BCOR and BCORL1 expression ranked in the top 5% expressing tumors in a panel of 2642 solid tumors. Unsupervised clustering on the expression profiles issued from this series identified a biologically homogeneous group of tumors, together with BCOR-rearranged sarcomas and characterized by an upregulation of genes involved in the cell cycle/proliferation pathways, morphogenesis/development, translation and spliceosome and a low immune infiltrate. With FLAG immunoprecipitation followed by mass spectrometry, YWHAE::NUTM2 oncoprotein was found to interact with EP300, CREBBP, A-RAF and C-RAF but not BCOR surprisingly, suggesting an indirect dysregulation. With EP300 immunoprecipitation followed by Western Blot, we confirmed the interaction. Furthermore, we also detect that YWHAE::NUTM2 disrupt EP300 interactions with BCOR (Figure 1).

Conclusion: Altogether, our RNA-sequencing findings support that YWHAE::NUTM2 and BCOR rearranged sarcomas might represent a common entity. While YWHAE::NUTM2 does not seem to interact directly with BCOR, it might regulates BCOR expression by disrupting its interaction with EP300. Consequently, it might lead to Non-Canonical PRC1.1 (Polycomb repressive complex) dysregulation and then promote tumorigenesis.

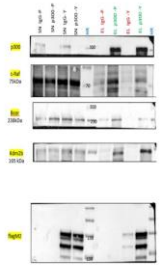


Figure 1. Western Blot results after EP300 immunoprecipitation. It confirms the interaction between YWHAE::NUTM2 and EP300, and show that YWHAE::NUTM2 seem to disrupt EP300 interactions with C-RAF, BCOR and KDM2B.

*SN: Supernatant; EL: Eluat; IgG: immunoglobulin G; P: pIRES plasmid vector ALONE; Y: Expressing FLAG-tagged plasmid pIRES-YWHAE::NUTM2; HR: High Range Protein Ladder.

(P 436) OUTCOME PROGNOSTICATORS IN ADOLESCENTS AND ADULTS WITH BONE SARCOMAS IN LOW-MIDDLE-INCOME COUNTRIES

Laboni Sarkar, MD[1]; Goutam Santosh Panda, D.M.[1]; Sushmita Rath, D.M.[1]; Nehal Khanna, M.D.[1]; Bharat Rekhi, MD DNB FRCPATH[1]; Siddhartha Laskar, M.D.[1]; Shripad D Banavali, MD[2]; Jyoti Bajpai, MD, DM[3], (1) Tata Memorial Hospital Mumbai, Mumbai, Maharashtra, India, (2) Tata memorial Centre, Mumbai, Mumbai, Maharashtra, India, (3) Tata Memorial Centre,Homibhabha National University,Mumbai,India, Mumbai, Maharashtra, India

Objective: Treating patients with bone sarcomas, including Ewing's Sarcoma (ES) and osteosarcoma (OGS) in Low-Middle Income Countries (LMIC) has multiple unique challenges. Social inhibitions and lack of awareness leads to high tumor burden and higher incidence of metastases at presentation. Inadvertent prior treatment by primary care physicians, high dropout rates and poor adherence compromises outcomes. We have aimed to identify prognostic factors including those specific to LMIC.

Methods: All adolescent and adult OGS and ES cases treated at Tata Memorial Centre, Mumbai, with the OGS-12 and Ewing's family of tumors-2001 [EFT-2001]) protocol from November 2011 to January 2021, and January 2013 to November 2018 respectively were prospectively analyzed. The OGS-12 protocol consists of doublets of doxorubicin, ifosfamide and cisplatin given sequentially for eight cycles in both neoadjuvant (NACT) and adjuvant (ACT) settings. The EFT-2001 protocol consists of a 12-month course of ifosfamide plus etoposide and vincristine, doxorubicin plus cyclophosphamide. Disease factors were symptom duration, lactate dehydrogenase (LDH), serum alkaline phosphatase (SAP), nutritional parameters, tumor size, presence and sites of metastases, pathological fracture and neurovascular bundle involvement. Treatment characteristics were treatment duration, protocol completion, prior treatment, febrile neutropenia (FN) and dose modifications. These were recorded from electronic medical records (EMR). The primary outcome was event-free survival (EFS). Secondary outcomes were overall survival (OS) and histological necrosis. Effect of covariates on histological response was estimated by logistic regression analysis and on survival using Cox proportional hazards analysis. Significant factors on univariate analysis were tested in multivariate analysis.

Results: =There were 1063 adolescents and adults aged >15 years with bone sarcomas, including 828 OGS cases and 235 ES cases. Males constituted the majority, 505 (62%) of OGS cases and 162 (69%) of ES cases respectively. The median age of presentation was 19 (range: 15-67) years. 256 (31%) OGS patients and 52 (22%) ES patients had metastatic disease. Most had high tumor burden and poor nutritional status, as described in Table 1. The median treatment duration was 40 (range: 36-61) weeks for OGS and 71 (range: 61-87) weeks for ES. 753 (91%) of OGS and 181 (77%) of ES cases completed the treatment protocol. 98 (12%) of OGS and 29 (9%) of ES cases had received prior treatment. Good histological necrosis (≥90%) was seen in 435 (55%) of 791 evaluable OGS patients. ≥90% necrosis was observed in 106 (48%) and ≥99% in 68 (31%) patients of 221 evaluable ES patients. 439 (53%) OGS patients and 103 (44%) of ES cases had FN, with 182 (22%) and 44 (19%) requiring dose modification, respectively. patients. At a median follow-up of 64.3 (range: 5-81 months), the median EFS ad OS of the OGS cohort was not reached, mean EFS and OS were 39 (95% CI: 5-61) months and 66 (95% CI: 8-91) months respectively. On univariable analysis, for both ES and OGS separately, the presence of baseline metastatic disease; tumor diameter >10cm; higher baseline SAP and LDH, absence of FN and failure to complete protocol and treatment duration >40 weeks were predictive of inferior EFS, as shown in Table 2. For OGS, baseline pathological fracture, necrosis < 90% and treatment duration >40 weeks were additional

predictive factors. For ES, necrosis < 99% and treatment duration >72 weeks were predictive. However, on multivariable analysis, only the presence of baseline metastases (HR: 4.1, p< 0.001); pathological fracture (HR 1.3, p=0.04), poor histological necrosis (HR 1.9, p=0.01), failure to complete protocol (HR 1.2, p=0.04), and prior treatment (HR 1.7, p=0.02) independently predicted inferior EFS. These factors were also predictive of inferior OS, as shown in Table 3.

Conclusion: This is the largest prospective analysis of bone sarcomas in India, with generalizable results for LMIC. Metastatic disease for both OGS and ES and pathological fracture for OGS were disease characteristics resulting in worse outcomes. Importantly, indicators of poor treatment compliance such as failure to complete the planned protocol and social challenges such as prior inadvertent treatment were also independently predictive of inferior outcomes, Future directions include development of a predictive model for prognosticators in ES and OGS that will be of great importance in improving outcomes in LMIC.

Table 1: Baseline Characteristics

	Osteosarcoma (OGS)		Ewing's sarcoma (ES)	
Demographics	**No. (%)**	**Median (IQR)**	**No. (%)**	**Median (IQR)**
Age		19 (15-71) years		20 (15-66) years
Gender				
Males	505 (62%)		162 (69%)	
Females	323 (28%)		73 (31%)	
Nutritional Parameters	**No. (%)**	**Median (IQR)**		**Median (IQR)**
Deficient albumin (<3.5g/dl)	43 (5.2%)		23 (9.8%)	
Anemia	613 (74%)		165 (70.4%)	
Iron Deficiency	524 (63.3%)		150 (63.8%)	
B12 Deficiency	274 (33.1%)		82 (34.9%)	
Folate Deficiency	88 (10.6%)		28 (11.9%)	
Malnourishment	85 (14%)		38 (16.2%)	
Disease Characteristics	**No. (%)**	**Median (IQR)**	**No. (%)**	**Median (IQR)**
Metastatic	256 (31%)		52 (22%)	
Symptom Duration		4.1 (0.5-12) months		3.8 (0.5-8) months
Elevated Lactate Dehydrogenase (>190 U/l)	621 (75%)		162 (69%)	
Elevated Serum Alkaline Phosphatase (>171U/l)	604 (73%)		103 (43%)	
Neurovascular involvement	83 (10%)		-	
Pathological fracture	107 (13%)		-	
Joint involvement	149 (19%)		-	
Tumor size		8.6 (5.2-14.2) cm		9.6 (5.2–11.6) cm
Treatment Characteristics	**No. (%)**	**Median (IQR)**	**No. (%)**	**Median (OQR)**
Histological Necrosis				
≥90%	435 (55%)		106 (48%)	
≥99%	240 (29%)		68 (31%)	
Treatment duration		40 (36-61) weeks		71 (61-87) weeks
Protocol Completion	753 (91%)		181 (77%)	
Prior Treatment	98 (12%)		29 (9%)	
Febrile Neutropenia	439 (53%)		103 (44%)	
Dose Modifications	182 (22%)		44 (19%)	

Table 2: Prognostic Variables of Significance: Osteosarcoma

	Parameters	Univariate		Multivariate	
		Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
OS	Metastatic disease	4.1 (3.7-4.8)	0.0001	3.3 (2.9-37)	0.001
	Tumor diameter >10cm	1.9 (1.6-2.1)	0.04	1.1 (1.0-1.3)	0.1
	Elevated baseline serum alkaline phosphatase	1.8 (1.6-2.0)	0.03	1.2 (1.0-1.6)	0.1
	Elevated serum lactate dehydrogenase	1.5 (1.1-1.7)	0.04	1.1 (0.9-1.3)	0.11
	Absence of febrile neutropenia	1.5 (1.2-1.9)	0.04	1.2 (0.8-1.4)	0.3
	Prior treatment	1.9 (1.7-2.2)	0.001	1.5 (1.4-1.7)	0.01
	Treatment duration >42 weeks	1.8 (1.6-2.1)	0.003	1.4 (1.2-1.7)	0.02
	Necrosis <90%	1.5 (1.1-1.7)	0.0001	1.3 (1.1-1.7)	0.003
EFS	Metastatic disease	3.9 (3.6-4.1)	0.0001	3.2 (2.9-3.5)	0.0004
	Tumor diameter >10cm	1.8 (1.2-2.1)	0.02	1.4 (1.3-1.6)	0.1
	Elevated baseline serum alkaline phosphatase	1.6 (1.4-1.9)	0.04	1.3 (1.1-1.6)	0.1
	Elevated serum lactate dehydrogenase	1.3 (1.1-1.6)	0.03	1.1 (0.9-2.1)	0.2
	Absence of febrile neutropenia	1.4 (1.2-1.5)	0.04	1.1 (0.8-1.2)	0.3
	Prior treatment	1.8 (1.7-1.9)	0.006	1.5 (1.3-1.7)	0.001
	Treatment duration >42 weeks	1.7 (1.5-1.9)	0.009	1.4 (1.2-1.6)	0.01

Table 3: Prognostic Variables of Significance: Ewing's Sarcoma

	Parameters	Univariate		Multivariate	
		Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
OS	Metastatic disease	3.9 (3.6-4.1)	0.0001	3.6 (3.2-3.7)	0.001
	Tumor diameter >10cm	1.8 (1.5-2.2)	0.03	1.1 (1.0-1.4)	0.1
	Elevated baseline serum alkaline phosphatase	1.7 (1.4-2.0)	0.03	1.3 (1.1-1.7)	0.1
	Elevated serum lactate dehydrogenase	1.5 (1.1-1.7)	0.04	1.1 (0.9-1.3)	0.11
	Absence of febrile neutropenia	1.3 (1.0-1.8)	0.02	1.1 (0.8-1.5)	0.8
	Prior treatment	1.8 (1.5-2.1)	0.001	1.6 (1.5-1.8)	0.01
	Treatment duration >72 weeks	1.9 (1.8-2.1)	0.001	1.3 (1.2-1.6)	0.01
	Necrosis <99%	1.9 (1.7-2.0)	0.0001	1.7 (1.6-1.9)	0.001
EFS	Metastatic disease	3.9 (3.6-4.1)	0.0001	3.2 (2.9-3.5)	0.0004
	Tumor diameter >10cm	1.6 (1.3-2.1)	0.04	1.3 (1.1-1.9)	0.1
	Elevated baseline serum alkaline phosphatase	1.8 (1.3-1.9)	0.04	1.2 (0.9-1.7)	0.2
	Elevated serum lactate dehydrogenase	1.2 (1.1-1.5)	0.03	1.0 (0.9-2.1)	0.3
	Absence of febrile neutropenia	1.3 (1.0-1.8)	0.04	1.1 (0.8-1.5)	0.4
	Prior treatment	1.9 (1.7-1.9)	0.002	1.6 (1.3-1.8)	0.001
	Treatment duration >72 weeks	1.8 (1.6-1.9)	0.008	1.6 (1.4-1.7)	0.01

(P 437) ULTRA-RARE UNDIFFERENTIATED SMALL ROUND CELL SARCOMA: HOW SHOULD WE TREAT THEM? AN ANALYSIS FORM THE GRACEFUL PROJECT

Emanuela Palmerini, MD, PhD[1]; Marco Gambarotti, MD[2]; Elizabeth A. Connolly, MBCHB MRCP[3]; Steven G. Dubois, MD, MS[4]; Salvatore Provenzano, MD[5]; Javier Martin-Broto, MD, PhD[6]; Antoine Italiano, MD,PhD[7]; Hanna Koseła-Paterczyk, MD PhD[8]; Nadia Hindi, MD, MSc[6]; Ravin Ratan, MD[9]; Palma Dileo, MD[10]; Vivek A. Bhadri, MD, PhD[11]; Robin L. Jones, BSc, MB BS, MRCP, MD[12]; Lianne M. Haveman, MD, PhD[13]; Giovanni Grignani, MD[14]; Stefania Kokkali, N/A, MD[15]; Virginia Ferraresi, MD[16]; Christian Rothermundt, MD[17]; Antonia Digklia, Dr Med[18]; Antonella Brunello, MD, PhD[19]; Juan Díaz-Martín, PhD[20]; Daniela Katz, MD, MHA[21]; Silvia Hofer, MD[22]; Angela M. Hong, MBBS, MMed, PhD, FRANZCR[23]; Davide Maria Donati, Sr., MD[1]; Angelo Paolo Dei Tos, N/A, MD[24]; Toni Ibrahim, BSc, MSc, MD, PhD[25]; Bruno Vincenzi, MD, PhD[26]; Silvia Stacchiotti, MD[27], (1) IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (2) Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (3) Chris O Brien Lifehouse, Sydney, New South Wales, Australia, (4) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA, (5) Adult mesenchymal tumours and rare cancers unit, Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Ital, Milano, Lombardia, Italy, (6) Medical Oncology Department, Hospital Universitario Fundación Jiménez Díaz and Villalba General Hospital, Madrid, Spain; Medical Oncology, Madrid, Madrid, Spain, (7) Institut Bergonié, Bordeaux, Aquitaine, France, (8) Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (9) The University of Texas MD Anderson Cancer Center, Houston, TX, (10) University College Hospital, London, England, United Kingdom, (11) Chris O'Brien Lifehouse, Sydney, New South Wales, Australia, (12) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (13) Princess Máxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands, (14) Candiolo Cancer Institute, FPO – IRCCS, Turin, Piemonte, Italy, (15) National and Kapodistrian University of Athens, Oncology Unit, 2nd Department of Medicine, HIPPOKRATIO General Hospital of Athens, Athens, Attiki, Greece, (16) IRCCS Istituto Nazionale Tumori Regina Elena, Rome, Lazio, Italy, (17) Cantonal Hospital St. Gallen, St. Gallen, Sankt Gallen, Switzerland, (18) Department of Oncology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland., Lausanne, Vaud, Switzerland, (19) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy, Padua, Veneto, Italy, (20) Institute of Biomedicine of Sevilla (IBiS), Virgen del Rocio University Hospital/CSIC/University of Sevilla, 41013 Seville, Spain., Seville, Andalucia, Spain, (21) Helmsley Cancer Center, Shaare Zedek Medical Center, Jerusalem Israel, Jerusalem, Yerushalayim, Israel, (22) University Hospital Zurich, Zurich, Zurich, Switzerland, (23) Sydney Medical School, The University of Sydney and Department of Radiation Oncology, Chris O'Brien Lifehouse, Sydney Australia., Sydney, New South Wales, Australia, (24) Department of Medicine, University of Padua School of Medicine, Padova, Italy, Padova, Veneto, Italy, (25) Istituto Ortopedico Rizzoli IRCCS, Osteoncology- Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (26) Università Campus Biomedico di Roma, Roma, Italy, Rome, Lazio, Italy, (27) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milano, Lombardia, Italy

Objective: Undifferentiated small round cell sarcomas (URCSs) represent a diagnostic challenge, and their optimal treatment is unknown. We aimed to report on the treatment and

outcome of patients (pts) affected by URCS within the an international study (GRACefUl Project; NCT05357768).

Methods: URCS pts treated from 1983 to 2019 at 31 worldwide sarcoma reference centres were retrospectively identified. Based on molecular assessment, cases were classified as follows: (1) CIC-rearranged round cell sarcomas, (2) BCOR::CCNB3-rearranged round cell sarcomas, (3) unclassified URCSs (uURCS), (4) other genomic alterations. Primary end-point was Progression-Free Survival (PFS) defined as the time of date of biopsy until first progression or death, whichever occurred first (not applicable for group 4). Secondary end-point was overall survival (OS), defined as the time from of date of biopsy until to death or last available follow-up.

Results: In total, 243 pts were retrospectively identified: 150/243 (61%) CIC-rearranged sarcoma (median age 32 years, range 7-86), 47/243 (19%) BCOR::CCNB3-rearranged (median age 17 years, range 5-91), 40/243(16%) uURCSs (median age 38 years, range 4-70), and 6/243 (2%) other genomic alterations: EWSR1-NFATC2 (n = 4), CIC-LEUTX (n = 1), FUS-NFATC2 (n = 1), respectively.
Clinical presentations and treatments are shown in Table 1. Surgery was performed in 187/231 (80.9%) of the patients, combined with radiotherapy in 72/231 (31.2%); chemotherapy was used in 199/232 (85.6%), with no difference among groups; Ewing regimen was the most frequently used in the different histotypes (Table 1).
Stage at diagnosis of 234 pts included in the survival analysis was localized/resectable in 132 (56.4%), advanced in102 (43.6%).
At a 39-month median follow-up (range 6-373), the 3-year progression-free survival (PFS) was 58.9% (95% CI 50-76) in BCOR::CCNB3 patients, 40.8% (95% CI 35-52) in CIC-rearranged sarcomas, and 61.8% (95% CI 52-80.0) in uURCs (p = 0.009). With the limitation of a small sample size, no significant different PFS for BCOR::CCNB3 pts treated with an Ewing regimen vs. those receiving an Osteosarcoma regimen was shown; similarly, no difference among CIC::rearranged sarcoma pts treated with a Ewing regimen vs. doxorubicin and ifosfamide combination was proven. Nonetheless, Ewing regimen compared with doxorubicin and ifosfamide seemed associated with better outcome in uURCS (p=0.046) (Table 2).
3-year OS (95%CI) was 94.9% (95% CI 88-100) in BCOR::CCNB3 patients, 41.4% (95% CI 34-51), in CIC-rearranged, and 71.7% (55.6-87.8) in uURCS (p=0.0005).

Conclusion: This analysis confirmed that URCS encompasses several distinct ultra-rare sarcomas which are treated with different approaches across institutions. Prospective studies are needed to better define the optimal treatment strategy in each URCS subtype, and in particular to understand whether there are subtypes in which an intensive Ewing regimen can improve the outcome.

Table 1. Demography and treatment

	CIC-rerranged (n=149)	BCOR::CCNB3 (n=47)	uURCS (n=40)	EWSR1::NFATC2 (n=4)	CIC::LEUTX (n=1)	FUS::NFATC2 (n=1)	p *
Demography							
Age, y, median (range)	32 (7-86)	17 (5-91)	38 (4-70)	56 (11-71)	69	57	<0.0001
Sex, F/M	74/75	8/38	17/23	4/0	0/1	1/0	0.001
Site, Soft tissue/Bone	142/8	14/32	36/4	1/3	1/0	0/1	<0.0001
Stage, Localized/Metastatic	70/75	35/12	26/14	4/0	0/1	1/0	0.002
Local treatment							
Surgery, n %	65/141 46	24/46 52	21/38 55	4/4 100	0/1	1/1	0.748
Surgery + RT, n %	48/141 34	11/46 24	12/38 32	0/4	1/1 100	0/1	
RT, n %	13/141 9	10/46 22	2/38 5	0/4	0/1	0/1	
No local treatment	15/141 11	1/46 2	3/38 8	0/4	0/1	0/1	
Chemotherapy							
Ewing regimen, n %	94/139 68	25/46 54	14/36 39	1/4 25	0/1	0/2	<0.0001
Dox-ifosfamide, n %	25/139 18	3/46 6.5	13/36 36	0/4	0/1	1/2 50	
Osteo regimen, n %	0/139	9/46 20	0/36	0/4	0/1	0/1	
Other, n %	2/139 1	2/46 4.5	2/36 6	0/4	0/1	0/1	
No chemotherapy, n %	18/139 13	7/46 15	7/36 19	0/4	0/1	0/1	

* Analysis only included CIC-rearranged, BCOR-rearranged and unclassified URCS (uURCS)

RT = radiotherapy; Dox = doxorubicin; Osteo = osteosarcoma

Table 2. 3-year Progression-Free survival (PFS) by treatment arm

		n	Treatment	3-y PFS	95%CI	p
CIC-rearranged	All	71	Ewing regimen	28.6%	17.3 - 40.0	0.487
		23	Dox-ifosfamide	40.6%	19.3 - 61.9	
	Advanced	35	Ewing regimen	11.4%	0.0 - 23.2	0.996
		12	Dox-ifosfamide	12.5%	0.0 - 33.7	
	Localized/ resectable	35	Ewing regimen	45.5%	28.1 - 63.0	0.318
		11	Dox-ifosfamide	70.1%	41.3 - 99.0	
BCOR::CCNB3	All	23	Ewing regimen	52.0%	28.7 - 75.3	0.237
		7	Osteo regimen	85.7%	59.8 - 100.0	
	Advanced	7	Ewing regimen	26.8%	0.0 - 68.7	NA
		1	Osteo regimen	100%		
	Localized/ resectable	16	Ewing regimen	60.3%	59.8 - 100.0	0.216
		6	Osteo regimen	83.3%	53.5 - 100.0	
uURCS	All	14	Ewing regimen	71.4%	47.8 - 95.1	0.046
		12	Dox-ifosfamide	37.5%	8.7 - 66.3	
	Advanced	5	Ewing regimen	20.0%	0.0 - 55.1	0.424
		4	Dox-ifosfamide	50.0%	1.0 - 99.0	
	Localized/ resectable	9	Ewing regimen	100%		0.003
		8	Dox-ifosfamide	29.2%	0.0 - 63,1	

RT = radiotherapy; Dox = doxorubicin; Osteo = osteosarcoma

(P 438) ALVEOLAR RHABDOMYOSARCOMA WITH "BURKITT-LIKE" MORPHOLOGY IS ASSOCIATED WITH HIGH AMPLIFICATION OF MYCL

Michael Arnold, MD PhD[1]; Wei Xue, PhD[2]; Sonja Chen, MD[3]; Archana Shenoy, MD[3]; Lisa a. Teot, MD[4]; Abha Gupta, MD MSc[5]; Suzanne Wolden, MD[6]; Roshni R. Dasgupta, MD MPH[7]; Rajkumar Venkatramani, MD[8]; Kelly Klega, MS, MLS(ASCP)[9]; Brian Crompton, MD[9]; Erin Rudzinski, MD[10]; Natalie B. Collins, MD, PhD[11], (1) Children's Hospital of Colorado/University of Colorado, Aurora, CO, (2) University of Florida, Gainsville, FL, (3) Nationwide Children's Hospital/The Ohio State University College of Medicine, Columbus, OH, (4) Boston Children's Hospital and Harvard Medical School, Boston, MA, (5) Princess Margaret Cancer Center, Toronto, ON, Canada, (6) Memorial Sloan Kettering Cancer Center, New York, NY, (7) Cincinnati Children's Hospital Medical Center, Cincinnati, OH, (8) Baylor College of Medicine / Texas Children's Hospital, Houston, TX, (9) Dana-Farber Cancer Institute, Boston, MA, (10) Seattle Children's Hospital/University of Washington, Seattle, WA, (11) Dana-Farber/Boston Children’s Cancer and Blood Disorders Center, Boston, MA

Objective: Alveolar rhabdomyosarcoma (ARMS) is a distinct histologic category of rhabdomyosarcoma, with approximately 80% of cases associated with FOXO1 gene fusions. Outcomes for patients with ARMS are strongly associated with the presence of FOXO1 gene fusions, and additional genetic markers are essential to better define the cohort of FOXO1 fusion negative ARMS.

Methods: We reviewed the histology of 306 diagnostic biopsies submitted to the recent Children’s Oncology Group (COG) trial ARST1431.

Results: Histologic review of biopsy specimens from ARST1431 identified two cases with ARMS histology (defined as tumor cells with uniform, round nuclei) as well as distinctive large nucleoli with coarse chromatin, amphiphilic cytoplasm and abundant karryorrhexis resembling Burkitt lymphoma. Both cases showed diffuse myogenin reactivity, confirming the diagnosis of ARMS, and both cases were negative for FOXO1 fusions by FISH testing. The cases arose in the perianal region or upper extremity, in a 2 and 1 year old patient, respectively. Additional sequencing studies identified high amplification (>50 copies) of the MYCL proto-oncogene in both cases.

Conclusion: We hypothesize that the distinctive phenotype of these two cases is driven by MYCL amplification. MYCL high amplification has been previously reported in one case of ARMS that was FOXO1 fusion negative and refractory to therapy. The previously reported case displayed similar histologic features to the cases we identified in ARST1431. These cases demonstrate that histologic features can be used to aid in identifying novel genetic signatures among cases of fusion negative ARMS. Clinical follow-up for ARST1431 will soon be available to further examine the outcome for this small but unique set of patients. However, identification of additional cases will be required to determine the clinical significance of this “Burkitt-like” histologic pattern with MYCL amplification.

(P 439) THE TETRAVALENT DEATH RECEPTOR 5 AGONIST INBRX-109 COMBINED WITH CHEMOTHERAPY IN EWING SARCOMA: PRELIMINARY DATA FROM A PHASE 1 STUDY

Rashmi Chugh, MD[1]; Sant P. Chawla, MD, FRACP[2]; Alexander Spira, MD, PhD, FACP[3]; Vivek Subbiah, MD[4]; Lee Hartner, MD[5]; Breelyn A. Wilky, MD[6]; Nehal Lakhani, MD, PhD[7]; Christopher Lieu, MD[8]; Ravin Ratan, MD[9]; Brianne O'Neill, PhD[10]; Vasily Andrianov, MD[10]; Emily C. Piccione, PhD[10]; James Kalabus, PhD[10]; Katelyn M. Willis, PhD[10]; Brendan Eckelman, PhD[10]; Patrick J. Grohar, MD, PhD[11], (1) University of Michigan Rogel Cancer Center, Ann Arbor, MI, (2) Sarcoma Oncology Center, Santa Monica, CA, (3) Virginia Cancer Specialists, Fairfax, VA, (4) Sarah Cannon Research Institute, Nashville, TN, (5) Abramson Cancer Center, Philadelphia, PA, (6) University of Colorado Cancer Center, Aurora, CO, (7) START Midwest, Grand Rapids, MI, (8) University of Colorado, Aurora, CO, (9) The University of Texas MD Anderson Cancer Center, Houston, TX, (10) Inhibrx, Inc, La Jolla, CA, (11) Children's Hospital of Philadelphia, Philadelphia, PA

Objective: Ewing sarcoma (EWS) and desmoplastic small round cell tumor (DSRCT) are rare, aggressive tumors that occur in children and adults and are frequently metastatic at diagnosis. Relapse is common, and few effective treatments are available. Irinotecan (IRI) and temozolomide (TMZ) are frequently used in the relapsed setting, but prognosis remains poor. INBRX-109 is a tetravalent, agonistic antibody targeting death receptor 5 (DR5), a proapoptotic TRAIL receptor. INBRX-109 is based on a single-domain antibody platform and is precisely engineered to achieve optimal balance of agonism and safety. INBRX-109 includes 4 DR5-binding domains to potently agonize DR5 through efficient receptor clustering and minimizes recognition by preexisting antidrug antibodies (ADAs), lessening the potential for hyperclustering and hepatotoxicity. In preclinical analyses, INBRX-109+IRI significantly increased antitumor activity in an EWS xenograft model compared with either agent alone and led to complete tumor regression in all treated mice. INBRX-109+IRI/TMZ is being explored in patients (pts) with EWS and DSRCT as part of an ongoing phase 1 study (NCT03715933). We present preliminary findings from this cohort.

Methods: This 3-part study is evaluating INBRX-109 in advanced/metastatic solid tumors. Part 1 (single-agent dose escalation) was completed. INBRX-109 was well tolerated, with no significant toxicities observed; a maximum tolerated dose was not reached, and a 3-mg/kg dose was chosen for further investigation. Part 2 (single-agent expansion) and part 3 (combination therapy expansion) are ongoing. In part 3, INBRX-109+IRI/TMZ was examined in pts with advanced/metastatic, unresectable EWS or DSRCT who had received 1-4 prior lines of treatment with ≥1 first-line chemotherapy regimen (cohort C3). INBRX-109 (1 or 3 mg/kg intravenously [IV]; day 1) was administered with IRI (50 mg/m^2/day IV; days 1-5) and TMZ (100 mg/m^2/day orally; days 1-5) every 21 days. Starting dose of INBRX-109 was 1 mg/kg in the first safety lead-in (cohort C3A) and 3 mg/kg in the second (cohort C3B). Intrapatient escalation from 1 to 3 mg/kg in cohort C3A occurred after safety review committee approval. IRI/TMZ could be stopped after ≥6 cycles at investigator discretion due to potential risk of accumulating toxicities; INBRX-109 was continued. The primary endpoint was safety; pharmacokinetics (PK), immunogenicity, and efficacy were secondary endpoints.

Results: As of data cutoff (May 1, 2023), 10 pts (EWS, n=7; DSRCT, n=3) had received INBRX-109 in cohort C3: C3A (1 mg/kg), n=7 (1→3 mg/kg, n=4); C3B (3 mg/kg), n=3. Five pts were still receiving treatment (C3A [3 mg/kg], n=2; C3B, n=3). Median age was 35 y (range, 21-71 y), and pts had received a median of 2 prior lines of therapy (prior IRI and/or TMZ, n=3).

Consistent with the known safety profile of IRI/TMZ, the most common adverse events (AEs) were diarrhea (any grade, n=5; grade ≥3, n=2), fatigue (n=5; n=0), and decreased platelet count (n=5; n=2). One pt had increased alanine aminotransferase (grade 1); no other liver-related AEs were reported. INBRX-109+IRI/TMZ demonstrated preliminary efficacy, with an overall response rate (ORR) of 60% (all confirmed partial responses [PRs]) and a disease control rate of 80%. Overall, 71% of pts with EWS (5 of 7) and 33% of pts with DSRCT (1 of 3) achieved PRs. Notably, a response was observed in 1 pt who had disease progression while receiving prior IRI or TMZ. Most pts with tumor shrinkage at first scan post treatment had further decreases in tumor size with continued treatment. Preliminary immunogenicity data suggest no impact of ADAs on PK.

Conclusion: INBRX-109+IRI/TMZ led to high response rates in pts with EWS (71%) and DSRCT (33%), with an ORR of 60% in cohort C3; no grade ≥2 hepatotoxicity or significant AEs leading to discontinuation were observed. Our findings support further evaluation of INBRX-109+IRI/TMZ in these tumors. Cohort C3C is now enrolling (n≈10) at the RP2D of 3 mg/kg. Updated data will be presented.

(P 440) LONG TERM OUTCOMES OF EWING SARCOMA OF THE FOOT AND ANKLE

Alexandra Arguello, MD; Mikaela Sullivan, MD; Steven Robinson, MBBS; Brittany Siontis, MD; Scott Okuno, M.D.; Thanh P. Ho, MD; Wendy A. Allen-Rhoades, N/A, MD; Peter S. Rose, MD; Matthew T. Houdek, MD, Mayo Clinic, Rochester, MN

Objective: Ewing sarcoma (ES) is one of the most common malignancies of the foot and ankle despite this being a rare location for the primary bone tumor. Standard of care involves chemotherapy and local control with surgery or radiotherapy. Surgical treatment of ES of the foot and ankle has historically involved amputation, though limb salvage options are available if a functional foot can be preserved. The aim of this study is to review our institution's management of Ewing sarcoma of the foot and ankle.

Methods: We reviewed 21 (16 male:5 female; mean age 20±14 years) patients with ES of the foot or ankle. The most common location of the tumor was the metatarsals (n=5) and distal fibula (n=4). All patients were treated with chemotherapy, most commonly VDC/IE in a neoadjuvant and adjuvant fashion following local control. Local control consisted of surgical resection (n=17) or definitive radiotherapy (n=4). Of the patients who underwent surgical resection, 12 were treated with an amputation (below knee, n=10; Syme, n=2). Limb salvage surgery included wide local excision (n=3) and ray resections (n=2). The mean follow-up was 10-years. The mean tumor necrosis on the resected specimens was 63% (range 0-100%). Five patients presented with metastatic disease which was treated with radiotherapy following consolidation chemotherapy. These patients were not included in the metastatic disease-free survival.

Results: Following treatment, the 10-year disease specific survival was 57%. Patients undergoing surgery for local control had improved 10-year survival compared to those treated with definitive radiotherapy (65% vs 25%, p=0.04). There was no difference in 10-year survival between patients treated with limb salvage and those with an amputation (48% vs. 64%, p=0.86). Disease recurrence occurred in 6 patients and included metastatic disease (n=5) and local progression and metastatic disease (n=1). The mean time to development of metastatic

disease was 2.5 years. The 5-year metastatic free survival was 59%. The case of local tumor progression occurred in the setting of previous definitive radiotherapy. Surgical complications occurred in 3 patients. Of the limb salvage patients, one underwent secondary transtibial amputation due to valgus deformity, ankle pain and limb length discrepancy.

Conclusion: Combined chemotherapy and definitive surgical management resulted in 100% local control and improved disease specific survival in the setting of Ewing sarcoma of the foot and ankle. Tumor location will largely dictate the ability to perform a limb salvage surgery, however if limb salvage is able to be done, it does not impact survival.

(P 441) LONG VERSUS SHORTER SCHEDULES OF VINCRISTINE, IRINOTECAN AND TEMOZOLOMIDE (VIT) FOR RELAPSED OR REFRACTORY EWING SRACOMA: A RANDOMISED CONTROLLED PHASE 2 TRIAL

Jie Xu, MD; Lu Xie, MD; Wei Guo, N/A, M.D.; Xiaodong Tang, M.D.; Xin sun, N/A, MD, Peking University People's Hospital, Beijing, Beijing, China (People's Republic)

Objective: The optimal dose schedule of vincristine, irinotecan, and temozolomide (VIT) in relapsed or refractory patients with Ewing sarcoma requires clarification.

Methods: We did this phase 2 randomized controlled trial at two hospitals. We included patients with relapsed or refractory Ewing sarcoma. They were randomly assigned (1:1) by random number table method to either shorter dx5 schedule (Irinotecan 50mg/m2/d D1-5, Vincristine 1.4mg/m2 D1) or protracted dx5x2 schedule (Irinotecan 20mg/m2/d D1-5,8-12, Vincristine 1.4mg/m2 D1,8) together with a fixed dose of temozolomide (100mg/m2/d D1-5) in both groups. Patients were treated every 3 weeks till progression or unacceptable toxic effects for up to eight cycles. The primary endpoint was objective response rate at 12 weeks (ORR12w). The secondary endpoint was progression-free survival (PFS), overall survival (OS) and safety.
The study was powered to detect a 30% improvement in the response rate from 20% of dx5 schedule to 50% of dx5x2 schedule (α=0.1, 1-β=0.9, one-sided test favoring dx5x2 schedule since the only difference of clinical importance was an improved response with the more prolonged but inconvenient schedule of dx5x2). A sample size of 30 patients per group (60 randomly assigned patients) was required to detect a significant improvement in ORR12w.

Results: Between May 21, 2020, and August 8, 2022, 24 patients were randomly assigned to dx5 schedule while 22 to dx5x2 schedule, respectively. Median follow-up was 10.7 months (IQR 9.7-13.9) in the dx5 group and 8.3 months (IQR 4.4-15.3) in the dx5x2 group. ORR12w was lower in dx5 group than in dx5x2 group (5 [20.8%] of 24 patients vs 12 [54.5%] of 22; p=0.019). There was no significant difference in PFS between the two groups (median PFS 2.3 months [95% CI 0.0-4.7] in dx5 group vs 4.3 months [2.7-6.0] in dx5x2 group; hazard ratio [HR] 0.956 [95% CI 0.84-1.09]; p=0.434). Also, there was no significant difference in OS (median OS 14.8 months [95% CI 12.0-17.6] vs 12.8 months [95% CI 6.9-18.7]; HR 0.957 [95% CI 0.81-1.12]; p=0.594). Patients in dx5 schedule reported more grade 3 and 4 adverse events (AEs) than dx5x2 schedule, including diarrhea/abdominal pain (23[23.7%] of 97 courses vs 8[8.4%] of 95 courses, p=0.005), vomiting/nausea (6[6.2%] vs 1[1.15%], p=0.035). Other common grade 3 and 4 AE (>5%) were similar between two schedules (p>0.05), including fatigue (24[24.7%] vs 14[14.7%]), leukopenia (6[6.2%] vs 8[8.4%]), neutropenia (5[5.2%] vs 8 [8.4%]), and anemia

(5[5.2%] vs 7[7.4%]). Unfortunately, the isolation policy of COVID-19 significantly restricted the travel of patients from their hometown to our hospitals. Given the relatively slow speed of recruiting, the sponsor stopped following support to our study.

Conclusion: Protracted dx5x2 VIT schedule showed superior efficacy and favorable tolerability compared with the shorter dx5 VIT schedule in patients with relapsed or refractory Ewing sarcoma.

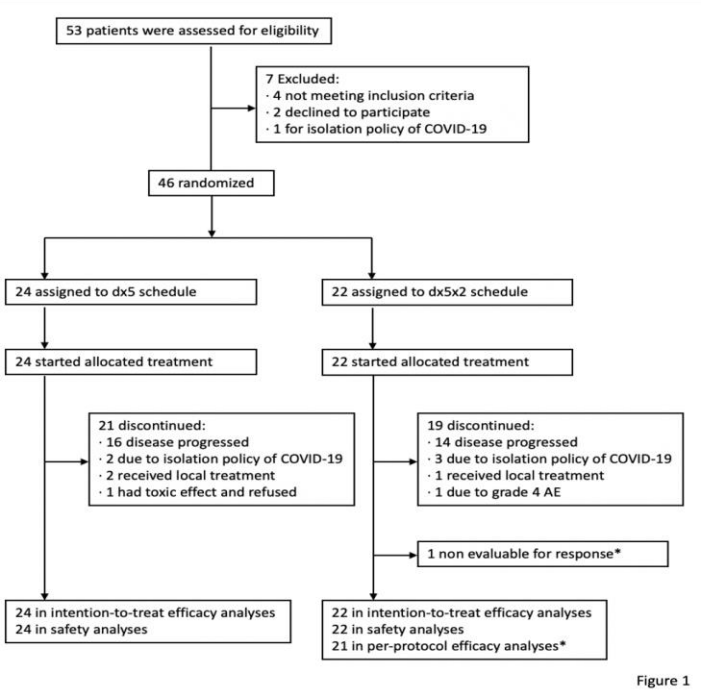


Figure 1

Table 1. Demographics and baseline characteristics.

Patients	**dx5 (*N* = 24)**	**dx5×2 (*N* = 22)**
Age (y; Mean ± SD)	16.5 ± 7.9	15.2 ± 6.3
Gender, *N* (%)		
Male	18 (75.0%)	15 (68.2%)
Female	6 (25.0%)	7 (31.8%)
Primary tumor location, *N* (%)		
Extremities	14 (58.3%)	14 (63.6%)
Axial skeleton	4 (16.7%)	6 (27.3%)
Other	6 (25.0%)	2 (9.1%)
Site of target and non-target lesions, *N* (%)		
Lung only	12 (50.0%)	7 (31.8%)
Other organs involved	12 (50.0%)	15 (68.2%)
Time from first diagnosis to enrollment, *N* (%)		
≤24 mo	20 (83.3%)	17 (77.3%)
>24 mo	4 (16.7%)	5 (22.7%)
Range (mo)	6-37	5-32
Lines of previous chemotherapy, *N* (%)		
1	20 (83.3%)	18 (81.8%)
≥2	4 (16.7%)	4 (18.2%)
Range (lines)	1-3	1-3
Relapsed or refractory disease before enrollment		
Relapsed disease	8 (33.3%)	9 (40.9%)
Refractory disease	16 (66.7%)	13 (59.1%)

Table 2. Efficacy of VIT in ITT population.

	dx5 ($n = 24$)	dx5×2 ($n = 22$)[a]
Response at 12 weeks		
Complete response	1 (4.2%)	1 (4.5%)
Partial response	4 (16.7%)	11 (50.0%)
Stable disease	6 (25.0%)	3 (13.6%)
Progressive disease	13 (54.2%)	7[a] (31.8%)
ORR at 12 weeks	20.8%	54.5%
ITT Progress-free survival		
KM median (mo)	2.3 (0.0–4.7)	4.3 (2.7–6.0)
ITT Overall survival		
KM median	14.8 (12.0–17.6)	14.6 (8.7–20.5)
Patients' status at last follow-up		
NED	4 (16.7%)	3 (14.3%)
AWD	5 (20.8%)	8 (38.1%)
DOD	15 (62.5%)	10 (47.6%)

Note: Data are *n* (%); % (95% CI) or months (95% CI).
Abbreviations: ORR, overall response rate; ITT, intention-to-treat population; KM, Kaplan–Meier; NR, not reached; NED, no evidence of disease; AWD, alive with disease; DOD, died of disease.
[a]One patient received only one cycle of dx5×2 and dropped out because of COVID-19 isolation policy. He failed to get any evaluation and was recorded as PD in ITT population.

Table 3. Grade 3/4 toxicities attributed to therapy ($n = 192$ courses).

Regimen	dx5 ($N = 97$)		dx5×2 ($N = 95$)	
Adverse event	*N*	(%)	*N*	(%)
Diarrhea/abdominal pain	23	23.7%	8	8.4%
Vomiting/nausea	6	6.2%	1	1.1%
Leukopenia	6	6.2%	8	8.4%
Neutropenia	5	5.2%	8	8.4%
Anemia	5	5.2%	7	7.4%
Thrombocytopenia	2	2.1%	2	2.1%
Hypokalemia	2	2.1%	3	3.1%
ALT increased	0	0%	2	2.1%
AST increased	0	0%	2	2.1%
Fatigue	24	24.7%	14	14.7%
Weight loss	3	3.1%	2	2.1%

(P 442) LONG-TERM OUTCOME AND RELAPSE PATTERNS IN EWING SARCOMA PATIENTS WITH EXTENSIVE LUNG/PLEURAL METASTASES AFTER A COMPLETE RESPONSE TO SYSYEMIC THERAPY

Jie Xu, MD[1]; Xin Zhi, MD[2]; Lu Xie, MD[1]; Xin sun, N/A, MD[1]; Xingyu Liu, MD[1]; Kuisheng Liu, M.D.[1]; Wei Guo, N/A, M.D.[1]; Xiaodong Tang, MD[1], (1) Peking University People's Hospital, Beijing, Beijing, China (People's Republic), (2) Peking University People's Hospital, beijing, Beijing, China (People's Republic)

Objective: Ewing sarcoma (ES) is sensitive to systemic therapy, including chemotherapy and anti-angiogenesis Tyrosine Kinase Inhibitors(aaTKIs). However, the prognosis of patients with metastatic disease remains poor. Recurrence or distant metastasis after a complete response (CR) or near-CR due to systemic therapy is not rare.There have been numerous clinical trials aiming at the prognosis of R2pulm group or recurrent/relapsed (RR) ES patients. However, for those who had extensive lung/pleural metastasis (L/Pmeta), then completely responded to chemotherapy and got whole lung irradiation as maintenance therapy, there is insufficient high quality data to answer the following questions: 1. How much portion of these patients could be cured at last? 2. If not, what are the relapse patterns in these former responders? To answer these questions, we carried out this retrospective study and reviewed ES patients from one institutes in China.

Methods: Patients
We conducted an Institutional Review Board-approved review of all patients with complete medical records diagnosed with ES and treated at Peking University People's Hospital and Peking University Shougang Hospital between 2014 and 2019. Inclusion criteria were: 1. Histologically-confirmed EWS; 2.Extensive L/Pmeta with no chance of radical radiotherapy (RT) or resection before multi-drug chemotherapy. Extensive L/Pmeta was defined when it fulfilled at least one of the following criteria: 1) Multiple pulmonary nodules in both lungs, and the largest nodule ≥ 0.5 cm (More than 5 nodules were defined as "multiple" in this study). 2) Pleural effusion and/or multiple pleural nodules; 3. Experiencing a complete response or nearly completely response (CR/nCR)] recorded and then confirmed after 6-8 weeks of multi-drug systemic therapy based on chemotherapy with or without aa-TKIs. CR was defined according to the Response Evaluation Criteria In Solid Tumors 1.1 (RECIST 1.1) guidelines. nCR was defined as each nodule < 3 mm.
Treatment
Alternating vincristine/doxorubicin/cyclophosphamide and ifosfamide/etoposide (VDC/IE) q2w were administered as first-line chemotherapy. Irinotecan-based regimens such as vincristine/irinotecan/temozolomide (VIT), or anlotinib, an aa-TKI plus vincristine and irinotecan was used as second-line chemotherapy in recurrent or refractory patients (NCT03359005 and NCT03416517).
WLI was given at the end for patients with first-line chemotherapy, or if all pulmonary lesions were < 3 mm in two consecutive CT scans for recurrent or relapsed patients. WLI was given by the 3DCRT technique. The median dose of prescribed radiation was 15 Gy/1.5 Gy/F, once per day; with the dose changing according to the hospital treatment policy from 12 Gy earlier to 15 Gy more recently. Notably, no patients received a boost dose.
Outcomes
EFS was defined from the date of first confirmation of CR/nCR to the first reported event (recurrence, progression, or death). Patients without an event were censored at the date of last follow-up. OS was defined from the date of first confirmation of CR/nCR to death or the date that they were last known to be alive. Chest computed tomography (CT) images were reviewed by two doctors to define the exact location of each recurrent lesion, compared with the primary

L/P lesion before chemotherapy, and the relapse pattern was then summarized according to the following criteria:
Pattern 1= All recurrent lesions completely coincide with the original metastatic lesions before chemotherapy (Figure 1)
Pattern 2= Some of the recurrent lesions coincide with the original lesions, but also with some new lesions
Pattern 3= All recurrent lesions are completely new lesions

Results: Patient Characteristics
17 pts and 21 cases of CR/nCR were finally analyzed. 2 patients experienced CR/nCR twice and 1 patient experienced CR/nCR a third time. Of these patients, 23.8% had primary extensive L/Pmeta, while 19.0% suffered L/Pmeta within 12 months of the initial diagnosis, and 57.1% after more than 12 months. The median time from extensive L/Pmeta to first confirmation of CR/nCR was 5.2 months. Median follow-up of surviving patients was 39.6 months.
Treatment details and survival
82.4% patients received WLI. At last assessment, 3 patients had no evidence of disease, 6 were still alive with disease and 8 had already died of disease (DOD). For 8 DOD patients, 4 died of recurrent lung/pleural metastases, while 4 of them died of distant metastases other than lung/pleual lesions. The median EFS and OS were 9.3 months and 37.5 months. The 2-year EFS was 19%. The 2-year OS was 70.6%. In all 17 patients, 12 were relapsed cases while the other 5 were initially metastatic cases. For the 12 relapsed cases, all of them joined clinical trial NCT03416517 (AVI for relapsed or recurrent EWS). The median EFS and OS were 6.5 months and 37.5 months. The 2-year EFS was 16.7% and OS was 66.7%.
Patterns of relapse
Lung/pleural relapse occurred in 71.4% (15/21) of CR/nCR cases. Most notably, in 9 of the 15 recurrent cases, the locations of all recurrent lesions exactly coincided with the original metastatic lesions (i.e. exactly in situ). This was the major relapse pattern; however, 4/9 cases also had distant metastases, while the other 5 cases had no lesions outside the lung/pleura. In 2 of the 15 cases with lung/pleural recurrence, some of the recurrent lesions were exactly in situ, but some new lesions in the lungs were also noted. Neither patient had distant metastases concurrently. For the last 4, all recurrent lung/pleural lesions were completely new, and two also had distant metastases as well. For the whole cohort, 42.9% had distant metastases outside of the L/P.

Conclusion: Survival of ES patients with extensive L/Pmeta remains poor, even after they have a complete response to systemic therapy. Recurrence exactly in situ is the major relapse pattern. WLI is not sufficient to prevent local recurrence in lung or pleura. More aggressive local treatment for metastatic lesions is warranted.

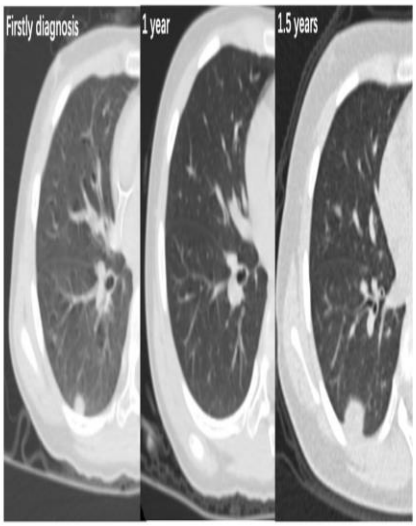


Fig. 1 A 9-year-old girl diagnosed with ES of right proximal humerus. She showed multiple pulmonary metastasis at diagnosis at 2016–7-19. At the end of 14 cycles of first-line compressed VDC-IE, CR was confirmed at 2017-6-30. Neither maintenance therapy nor WLI were given. Half a year later, exactly relapse in situ was diagnosed at 2017–12-18

Table 1 Demographics and baseline characteristics (21 CR/nCR in 17 patients[a])

Patients	Number	%
Age (years; Mean)	16 (range 4 – 39)	-
Sex		
Male	12	70.6%
Female	5	29.4%
EWSR-ETS translocation		
No	0	0
Yes	13	76.5%
Not available	4	23.5%
Primary site of the tumor		
Axial[b]	6	42.9%
Extremities	8	57.1%
Time from initially diagnosis of ES to extensive L/PMeta		
0	5	23.8%
< 12 months	4	19.0%
> 12 months	12	57.1%
Chemotherapy regimen before CR/nCR		
VDC-IE	5	23.8%
VIT	3	14.3%
AVI	13	61.9%
Time from L/PMeta to CR/nCR (months; Mean)	5.2	-
Lesions other than L/PMeta		
none	17	81.0%
Primary bone lesion	4	19.0%

[a] Two patients experienced CR/nCR twice and one experienced CR/nCR a third time

[b] No primary mediastinal EWS was included

Table 2 Maintenance therapy and survival after CR/nCR

Baseline characteristics				From meta to CR/nCR				Time from CR/nCR to the following conditions (months)			
No	Age	Time to meta	Baseline metastases[a]	Regimen before CR/nCR	Time to CR/nCR	WLI	Maintenance therapy	Status at last follow-up[b]	Last follow up	Local recurrence or metastatis	Death
1	8y	8.9	L	AVI	1.7	Yes	VI	DOD1	-	12.4	30.0
2	21y	10.3	P	AVI	3.6	Yes	CTX + Celecoxib	DOD2	-	6.2	37.5
3	21y	11.7	L	AVI	4.0	Yes	AVI	NED	38.8	-	-
4	23y	15.2	L	AVI	6.3	Yes	VI	AWD	33.4	3.5	-
5	36y	41.7	L	AVI	7.0	Yes	AVI	AWD	32.3	17.2	-
6	12y	8.5	L + P	AVI	5.7	Yes	A	DOD2	-	2.6	22.0
7	18y	21.7	L + P	AVI	5.8	Yes	VI + olaparib	DOD2	-	6.6	14.4
8	8y	18.7	L	AVI	2.8	Yes	A	AWD	33.0	6.5	-
9	39y	29.9	L	AVI	4.2	Yes	AVI	AWD	28.6	15.9	-
10	8y	0.0	L	VDC-IE	3.1	No	CTX + Celecoxib	AWD	63.8	31.5	-
	11y	14.7	L	AVI	7.5	Yes	A	AWD	24.8	22.8	-
11	13y	0.0	L	VDC-IE	6.2	No	No	DOD1	-	7.5	26.4
	15y	23.5	L	VIT	3.5	No	No	DOD1	-	4.1	5.6
12	4y	0.0	L	VDC-IE	4.1	No	No	AWD	57.7	12.9	-
	6y	17.1	L	IE-VI	6.5	No	CTX + Celecoxib	AWD	38.3	17.8	-
	8y	41.4	L	VIT	9.8	No	No	AWD	10.7	8.7	-
13	24y	0.0	L	VDC-IE	5.4	No	No	DOD1	-	9.3	16.8
14	28y	15.3	L	AVI	4.6	Yes	VIT	DOD1	-	2.4	17.2
15	30y	19.7	L	AVI	6.4	Yes	AVI	NED	33.2	-	-
16	8y	18.9	L	AVI	4.1	Yes	VI	DOD2	-	2.0	12.8
17	5y	0.0	P	VDC-IE	6.2	Yes	CTX + Celecoxib	NED	35.4	-	-

Time to meta: From initial diagnosis of EWS to the experience of ExL/PMeta (months)

Time to CR/nCR: From the delivery of first dose of chemotherapy drug to CR/nCR (months)

WLI Whole lung irradiation, *AVI* Anlotinib + Vincristine + Irinotecan, *VIT* Vincristine + vincristine + irinotecan, *VDC-IE* Vincristine + doxorubicin + cyclophosphamide / Ifosfamide + etoposide, *A* Anlotinib, *VI* Vincristine + irinotecan, *NED* None evidence of disease, *AWD* Alive with disease, *DOD* Dead of disease

[a] L = only lung metastases only; P = only pleural effusion/nodules; L + P = both lung and pleural effusion/nodules

[b] *DOD1* Died of lung/pleural metastases, *DOD2* Died of other reasons regarding to EWS

Table 3 Relapse pattern after CR/nCR

No	Whole lung irradiation	Time from CR/nCR to lung/pleural recurrence	Pattern of recurrence	Distant metastasis
1	Yes	12.4	1	No
2	Yes	6.2	1	Yes
4	Yes	3.5	3	No
5	Yes	-		Yes
6	Yes	2.6	1	Yes
7	Yes	6.6	1	Yes
8	Yes	-		Yes
9	Yes	15.9	3	Yes
10	No	31.5	3	Yes
	Yes	22.8	1	No
11	No	7.5	1	No
	No	4.1	2	No
12	No	12.9	1	No
	No	17.8	3	No
	No	No		Yes
13	No	9.3	2	No
14	Yes	2.4	1	No
16	Yes	2.4	1	Yes

Definition of each pattern according to the locations of recurrent lung/pleural lesions

1 = All recurrent lesions completely coincide with the original metastatic lesions before chemotherapy

2 = Some of the recurrent lesions coincide with the original lesions, but also with some new lesions

3 = All recurrent lesions are completely new lesions

(P 443) SURGICAL RESECTION OF EXTREMITY EWING'S SARCOMA BASED ON POST-CHEMOTHERAPY TUMOUR VOLUME RESULTS IN SAFE MARGINS

Patrick Qi Wang, MD, MSc, FRCSC[1]; Caroline D. Cristofaro, MD[1]; Nicholas Eastley, MBChB, BMedSci, FRCS (Tr & Orth), PhD[1]; Meshal Alfaraidy, MBBS, FRCSC[1]; Peter Ferguson, MSc., MD[1]; Jay Wunder, M.Sc, MD[1]; Kim Tsoi, MD, PhD[1]; Anthony Griffin, MSc[2], (1) University of Toronto, Toronto, ON, Canada, (2) Mount Sinai Hospital, Toronto, ON, Canada

Objective: Neoadjuvant chemotherapy is an integral part of the multimodal treatment approach to Ewing's Sarcoma. While chemotherapy may help reduce the tumour volume, the consensus amongst clinicians is that the entire pre-treatment tumour volume should still be resected during surgery to account for any residual, viable, and infiltrative malignant cells. However, this can lead to increased surgical morbidity and functional deficits. Therefore, this study investigated the safety of performing surgical resection for Ewing's Sarcoma based on post-chemotherapy tumour volumes.

Methods: A single centre retrospective review from a prospectively collected database between 1989 and 2019 was performed. All patients who underwent limb salvage resection of Ewing's sarcoma were included. Patients who underwent radiation therapy or initially presented with local recurrence were excluded. Outcomes of interest were surgical margins along with local recurrence-free and metastatic disease-free intervals, and overall survival. Survival analysis was performed using the Kaplan-Meier technique. A Cox-Regression analysis was performed to identify independent variables that affected the survival analysis.

Results: All 40 patients who were eligible for analysis underwent limb salvage resection based on post-chemotherapy MRI (magnetic resonance imaging) planning. The mean age was 26 years (range 13 to 64 years) with 17 males and 23 females. Tumour locations included the femur (n=19), tibia (n=8), scapula (n=5), humerus (n=5), fibula (n=2), and ulna (n=1). Four patients initially presented with pathologic fracture and four with metastatic disease. Tumour size decreased following chemotherapy in 82% of patients and increased in 18%. Tumour necrosis ≥ 90% was observed in 55% of patients. Twenty-five patients underwent additional postoperative chemotherapy. Complications were observed in 25% of patients including superficial wound infection (n=3), deep infection requiring debridement (n=1), nerve palsy (n=2), allograft fracture (n=1), acute soft tissue flap reconstruction failure (n=1), endoprosthesis bushing wear (n=2). The mean final follow-up was 87 months (range two to 328 months). Surgical margins were negative in all but one patient who had micro-positive margins. Local recurrence was seen in three of 40 patients (8%). Eight of 36 (22%) patients, who initially presented with isolated disease, developed metastatic disease. The five-year local recurrence-free, metastatic disease-free, and overall survival was 91.1%, 75.5%, and 71.8% respectively. Patient age, sex, tumour location, tumour size, response to chemotherapy, and pathologic fracture were not statistically significant for outcomes in the multivariate analysis.

Conclusion: The results suggest that the resection of extremity Ewing's sarcoma based on post-chemotherapy tumour volume results in an oncologic outcome comparable to those based on pre-chemotherapy tumour volume. Although further prospective comparative studies are required, the current findings suggest safe surgical margins can be achieved with this approach, potentially allowing for improved soft tissue coverage and functional outcomes.

(P 444) TWO CASES OF BRAIN METASTASES OF CD99 POSITIVE SMALL ROUND BLUE CELL TUMORS

Anderson B. Collier, III, MD[1]; Jennifer Cox, MD[2], (1) Nemours Children's Health , Jacksonville, FL, (2) University of Mississippi Medical Center, Jackson, MS

Objective: CD99 positive small round blue cell tumors (Ewing sarcoma) can occur in and metastasize to essentially all parts of the body, highlighting our lack of understanding of the cell of origin. While locations such as the lung and bone are common, we describe 2 cases of brain metastases of CD99 positive small round blue cell tumors. We review 3 large pediatric case series to highlight the rarity and propose an area of future investigation.

Methods: The charts of 2 patients treated at the authors' institution were reviewed. A literature search was conducted using PubMed.

Results: Case 1 was an African American patient who presented with widely metastatic CD99 positive, WT-1 negative small round blue cell tumor. EWSR1 break apart was negative. Initial brain MRI was normal. The disease responded to vincristine, doxorubicin, cyclophosphamide alternating with ifosfamide/etoposide (VDC/IE) and radiotherapy. The patient's disease progressed 7 months off therapy. The patient received 13 cycles of vincristine, irinotecan, and temozolomide (VIT) with disease stabilization until brain metastases were detected on scan for changes in personality and headaches. Radiotherapy followed by cabozantinib was administered. The systemic disease stabilized with only minimal growth of brain metastases for 1 year until progression of systemic disease. Therapy was discontinued; the patient died 6 months later, 4 years after diagnosis. Case 2 was a Caucasian patient who presented with a localized distal femur CD99 positive small round blue cell tumor with EWSR1-ERG translocation. The patient received 16 cycles of VDC/IE with surgery and radiotherapy. Fifteen months off therapy, a pulmonary relapse was treated with 16 cycles of VIT with good response and resection of disease. Three months later, recurrence was identified in the same lobe of the lung. The patient received 13 cycles of vincristine, topotecan, and cyclophosphamide, with good response, and resection. Three months later, a third pulmonary recurrence was discovered in the same lobe. The patient underwent 2 cycles of high dose ifosfamide, with good response, and a complete lobectomy. While recovering, 2 intraparenchymal brain metastases were discovered on imaging for altered mental status. The patient received radiotherapy and soon thereafter developed progressive systemic disease. Palliative radiotherapy and cabozantinib were administered. The patient died of progressive systemic disease 2 months after brain metastases, 5 ½ years after diagnosis.

Conclusion: Intraparenchymal brain metastases not associated with direct extension from bone metastases are rare in Ewing sarcoma. From three of the largest pediatric series, 27 out of 885 Ewing sarcoma patients experienced brain metastases (3%). All but one patient died with a reported median survival of 2.7 months from diagnosis of brain metastases. Brain was the only site of metastatic disease in only 3 cases, all from the oldest report. Translocation data is not reported, so the effect, if any, of translocations cannot be determined. Neither of our 2 cases had an EWSR1-FLI translocation, which highlights the need to continue to evaluate the differences in clinical behavior of CD99 positive small round cell sarcomas with various translocations to determine if specific translocations have higher incidence of brain metastases. This data may help inform monitoring strategies and treatment if a subset of patients with systemic metastases are at increased risk of metastases to the brain. References Parasuraman S, et.al. "Brain Metastases in Pediatric Ewing Sarcoma and Rhabdomyosarcoma: The St. Jude Children's Research Hospital Experience." J Pediatr Hematol Oncol 1999:21;370-377. Paulino

AC, et.al. “Brain Metastasis in Children with Sarcoma, Neuroblastoma, and Wilms’ Tumor.” Int J Radiation Oncology Biol. Phys. 2003:57;177-183. Trigg ME, et.al. “The Frequency of Isolated CNS Involvement in Ewing’s Sarcoma.” Cancer 1982:49;2404-2409.

(P 445) ISOTOXIC RADIOTHERAPY DOSE ESCALATION IN UNRESECTABLE PEDIATRIC EWING SARCOMA: A DOSIMETRIC FEASIBILITY STUDY

Sean A. Koerner, MD, MS; Abdelhamid Saoudi, PhD, MCCPM; Adam C. Olson, MD, MS, UPMC Hillman Cancer Center, Pittsburgh, PA

Objective: Radiotherapy (RT) is the definitive local therapy for unresectable localized or metastatic Ewing sarcoma (ES). A recent phase III trial testing standard-dose radiotherapy (SDRT) versus dose-escalated radiotherapy (DE-RT) showed improved local control (LC) with DE-RT. We hypothesized that utilization of a simultaneous integrated boost (SIB) to gross disease could enable isotoxic DE-RT for pediatric ES patients receiving definitive radiation therapy without increasing the number of radiation fractions, and that feasibility of this approach could support a phase I trial.

Methods: We retrospectively identified patients treated from 2012-2022 with pathologically confirmed ES, treated with definitive RT to a prescribed dose of 50.4 Gy to 55.8 Gy in 1.8 Gy fractions after induction chemotherapy. Patients who received surgery with gross residual disease were included. Standard target volumes included the pre-chemotherapy soft tissue and osseous extent for PTV1 (45 Gy in 1.8 Gy fractions), the post-chemotherapy soft tissue involvement and pre-chemotherapy osseous extent for PTV2 (5.4-10.8 Gy in 1.8 Gy fractions). The pre-chemotherapy osseous involvement was the subvolume selected for DE-RT (PTV3). OAR dose constraints were primarily adopted from AEWS1031. In addition, we contoured cauda equina and sacral plexus and converted AEWS1221 dose constraints to an isotoxic dose equivalent in 28 or 31 fractions ($\alpha/\beta = 2$). Isotoxic DE-RT was assessed at prespecified levels of 58.8 Gy, 67.2 Gy, and 75.6 Gy in 28 fractions or 65.1 Gy, 74.4 Gy, and 83.7 Gy in 31 fractions, defined as levels 1, 2, and 3, respectively. OAR constraints were prioritized over SIB dose escalation; dose escalation success was defined as V95% ≥ 95% of target volume while respecting constraints. We recorded DE-RT level achieved, OAR D5cc/D3cc and D0.03cc, DE-RT target volume, and conformity index (CI), gradient index (GI) with respect to adjacent dose-limiting OAR (cauda equina, sacral plexus, and spinal cord), and homogeneity index (HI) for original RT and DE-RT replan levels in each patient. RT planning was performed in Eclipse version 15.6 (Varian, Palo Alto, CA), and differences in CI, GI, and HI between original RT and DE-RT were assessed with matched-pairs t-test ($\alpha = 0.05$) in SPSS v27.0 (IBM, Armonk, NY).

Results: We identified ten patients meeting inclusion criteria, with a median age of 11 years (IQR 7-16). Unresected or residual ES was in the pelvis in five patients, lumbar spine and paraspinal musculature in three patients, and cervical/thoracic spine and paraspinal musculature in two patients. Two patients underwent partial resection prior to RT, and all patients received induction chemotherapy. Seven patients were successfully replanned to level 3 DE-RT, one to level 2, and one to level 1; one patient could not achieve DE-RT replan due to OAR (spinal cord) within the target volume. Median OAR and target doses at the highest DE-RT level achieved are reported in Table 1. Gradient indices with respect to each critical OAR were significantly increased with DE-RT at all dose levels excepting level 1 sacral plexus (Table 2). DE-RT replans displayed decreased homogeneity and increased conformity with respect to

the SIB volume as compared to prescribed plans (Table 2). Median DE-RT target volume was 20.03cc (IQR 7.4-44.4).

Conclusion: Isotoxic DE-RT using SIB was feasible for the majority of pediatric ES patients. DE-RT using SIB resulted in elevated GI, increased conformity, and decreased homogeneity, and safe delivery would require closely monitored image guided RT. A phase I trial of isotoxic dose escalation using our prespecified dose levels is planned.

Target	**DE-RT Dose**	**OAR Constraint**
Gross Disease	74.8 (69.5-83.9)	-
OAR		
Spinal Cord	49.15 (16.7-50.3)	$D_{0.03cc} < 50.4$
Cauda Equina	45.4 (10.4-50) 55.1 (33.7-55.8)	$D_{5cc} < 51.8$ $D_{0.03cc} < 56$
Sacral Plexus	9.05 (3.4-45.6) 51.8 (23.5-54.1)	$D_{3cc} < 51.8$ $D_{0.03cc} < 56$
Rectum	27.6 (4.2-27.6)	$D_{100\%} < 45$
Small Bowel	7.9 (4.3-8.34)	$D_{75\%} < 45$
Bladder	12.3 (3.6-12.3)	$D_{100\%} < 45$

Table 1. Median DE-RT target volume and OAR doses with planning constraints. Target and OAR dose reported in Gy with IQR.

	Rx	Level 1		Level 2		Level 3	
Homogeneity Index	3.9	6.7	*0.091*	11.8	***0.006***	15.2	***0.002***
Conformity Index	0.09	0.69	***<0.001***	0.75	***<0.001***	0.70	***<0.001***
Gradient Indices							
Spinal Cord	2.0	16.7	***0.009***	35.7	***0.002***	55.3	***0.014***
Cauda Equina	4.01	15.6	***0.015***	28.9	***0.008***	35.6	***0.004***
Sacral Plexus	5.9	15.2	*0.061*	30.9	***0.004***	41.4	***0.002***

Table 2. Comparison of dosimetric parameters of prescribed RT and DE-RT replan levels. Homogeneity, conformity, and gradient (Gy/cm) indices compared from prescribed plan to DE-RT replan at each level; paired t-test *p*-values are italicized.

(P 446) SYNERGISTIC TARGETING OF KIF11 AND AURKA TO IMPROVE OUTCOMES FOR EWING SARCOMA

Soumya M. Turaga, PhD[1]; Vikalp Vishwakarma, PhD[2]; Stacey Hembruff, MS[2]; Benjamin Gibbs, BS[2]; Priya Sabu, MD[2]; Rajni Puri, PhD[2]; Harsh Pathak, PhD[2]; Glenson Samuel, MD[2]; Andrew Godwin, PhD[2], (1) University of Kansas Medical Centre, Kansas City, KS, (2) KUMC, Kansas City, KS

Objective: Ewing sarcoma (EWS) is a rare and aggressive pediatric bone sarcoma. The pathological EWS/ETS gene fusion results in a driver oncogenic transcription factor, that remains undruggable. Standard of care treatment options for these children and young adults consists of multiple cycles of chemotherapy that often lead to toxicity, secondary malignancies, and co-morbidities. Although chemotherapy has improved the overall survival (OS) for localized disease, patients with metastatic disease have poor prognosis with less than 30% OS. To identify new therapies for this neglected disease, we conducted in silico and drug re-purposing screens (PMID: 27863422; 31025088). We identified proteins essential for mitotic spindle formation and cell cycle progression, including Kinesin Family Member 15 (KIF15), its binding partner, Xklp2 (TPX2), and Aurora Kinase A (AURKA) which are upregulated by EWS-FLI1, the primary oncogenic driver of EWS. Importantly, KIF15/TPX2 cooperates with KIF11 (Eg5), a molecular motor protein which cross-links anti-parallel microtubules in the mitotic spindle to maintain spindle bipolarity. We hypothesize that targeting key and redundant proteins essential for mitotic progression would provide a therapeutic vulnerability for Ewing sarcoma by inducing G2/M arrest and mitotic catastrophe. Given the lack of clinical grade KIF15 inhibitors, we targeted the protein upstream by inhibiting AURKA (VIC-1911; VITRAC Therapeutics) to block phosphorylation of KIF15S1169 and its subsequent targeting to the spindle. Thus we assessed co-targeting of KIF11 (SB-743921) and AURKA (VIC-1911) to exploit the chances for better efficacy, decreased toxicity, and reduced development of drug resistance. To advance our hypothesis, we evaluated the drug combination for synergy in vitro and tumor efficacy in vivo using EWS models

Methods: Drug Synergy assay:500 cells/well were plated in triplicates in 96-well plates in 8x8 matrix and incubated for 72 hours post drug treatment and cell viability was assessed by CellTiter-Glo reagent and synergy was calculated using SynergyFinder tool.
Cell cycle analysis: Cells were harvested and fixed in 70% ethanol overnight, post-drug treatment and then stained with FxCycle™ PI/RNase Staining Solution. DNA content was measured using the Attune™ NxT flow cytometer.
Capillary Western Blot (Wes): ProteinSimple Wes System was used to determine protein expression.1:50 dilution was used for primary antibodies.
Tumor xenograft and drug treatment studies: Six-week-old female NSG mice were inoculated subcutaneously with a suspension of TC-71 cells (2 × 106). Mice were randomized into four groups as follows: 1) Vehicle control; 2) SB-743921 only (2.5 mg/kg), 3) VIC-1911 (37.5 mg/kg), and 4) combination of SB-743921 and VIC-1911.

Results: The top fifteen genes that we identified to be essential for EWS progression are listed (Figure 1A). Further, in silico analysis of RNA expression data of solid cancers indicates that KIF15 expression levels are the highest in EWS and that KIF11 expression is the second highest in EWS after synovial sarcomas (Figure 1B). SB-743921 and VIC-1911 were tested across different EWS fusion bearing cells namely EWS-FLI Type II (SKES-1), EWS-FLI Type III (CHLA-258), and EWS-ERG (COG-E-352), and we observed synergistic drug activity with the combination treatment irrespective of fusion type (Figure 2). Combination treatment led to an increase in G2/M arrest and apoptosis (Figure 3A). Quantification of differences in cell-cycle

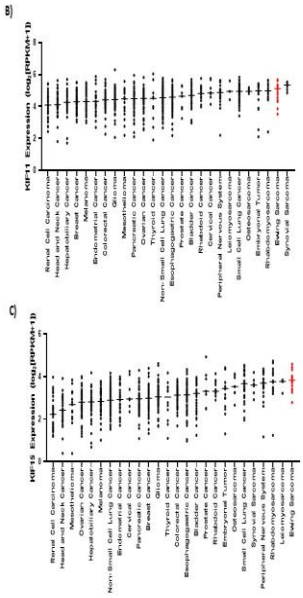
phases indicate a greater percentage of cells in G2/M phase for combination treatment of VIC-1911 (10 nM) + SB-743921 (2.5 nM) (53.1%) compared to single drugs [VIC-1911, 10 nM (34.3%), SB-743921, 5 nM (43.7%)], or the untreated control (29.2%). Wes analysis was done to determine protein expression following drug treatment and we observed increases in 1) KIF11 and AURKA expression in the combination group, and 2) c-PARP and p-KIF11Thr926 levels indicating apoptosis and delay in mitotic exit (Figure 3B). In vivo mouse xenograft studies using TC-71 demonstrate complete tumor regression with the combination treatment (Figure 4A) and significant effect on overall survival of the mice compared to control and single drug treatment groups (Figure 4B).

Conclusion: We have identified a novel combination of mitotic inhibitors targeting KIF11 and KIF15 via AURKA that is highly synergistic in inhibiting the growth of an aggressive tumor such as Ewing sarcoma. Our findings are highly relevant, timely and clinically translatable given the lack of proper therapies for this rare and orphaned disease

Figure 1: In silico screening identifies mitotic proteins essential for Ewing Sarcoma progression

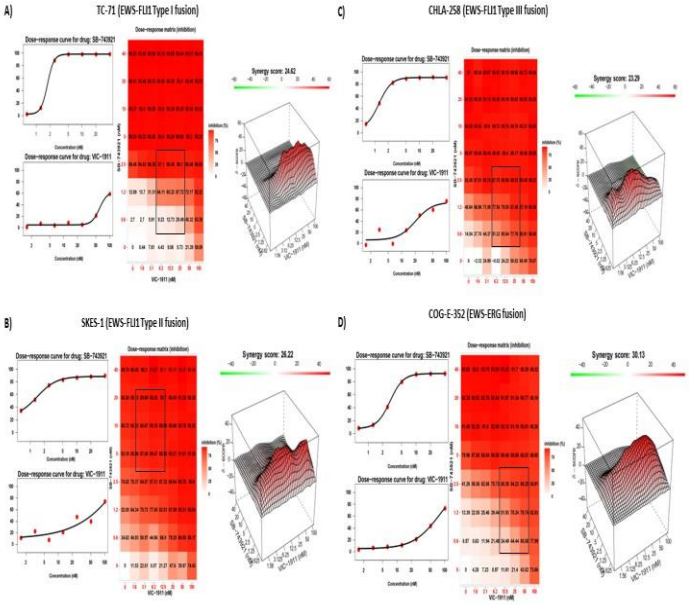
A)

Gene rank	Gene ID	Gene symbol	Name	Pathway
1	56992	KIF15	Kinesin family member 15	Motor proteins
2	1058	CENPA	Centromere protein A	Mitosis, chromosome segregation and cytokinesis
3	7153	TOP2A	Topoisomerase (DNA) II alpha 170 kDa	Platinum drug resistance
4	5502	PPP1R1A	Protein phosphatase 1, regulatory (inhibitor) subunit 1A	Adrenergic signaling
5	51361	HOOK1	Hook microtubule- tethering protein1	Vesicle trafficking
6	6790	AURKA	Aurora kinase A	Oocyte meiosis
7	5733	PTGER3	Prostaglandin E receptor 3 (subtype EP3)	Calcium signaling
8	2619	GAS1	Growth-arrest-specific1	Membrane trafficking
9	23308	TMEM194A	Transmembrane protein 194A	Nuclear envelope stiffness
10	1612	DAPK1	Death-associated protein kinase 1	Autophagy
11	29028	ATAD2	ATPase family, AAA domain containing 2	Transcriptional coactivator
12	783	CACNB2	Calcium channel, voltage dependent beta 2 subunit	MAPK signaling pathway
13	9787	DLGAP5	Discs, large (Drosophila) homolog-associated protein 5	Centrosome and spindle formation
14	22974	TPX2	TPX2, microtubule-associated, homolog (Xenopus laevis)	Regulation of kinetochore-microtubule interactions
15	2956	MSH6	MutS homolog 6	Mismatch repair



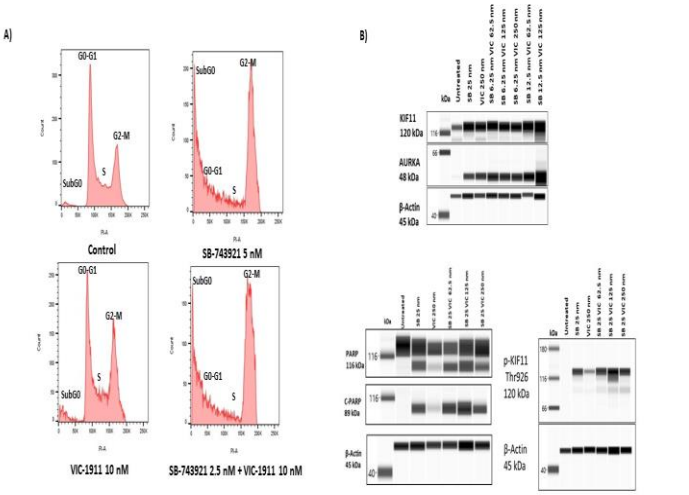
A) List of top-15 genes identified to be essential for EWS progression. Depmap portal was used to access the RNA-expression data of B) KIF11 and C) KIF15 across different cancer cell lines.

Figure 2. Synergistic inhibition of EWS growth by VIC-1911 and SB-743921



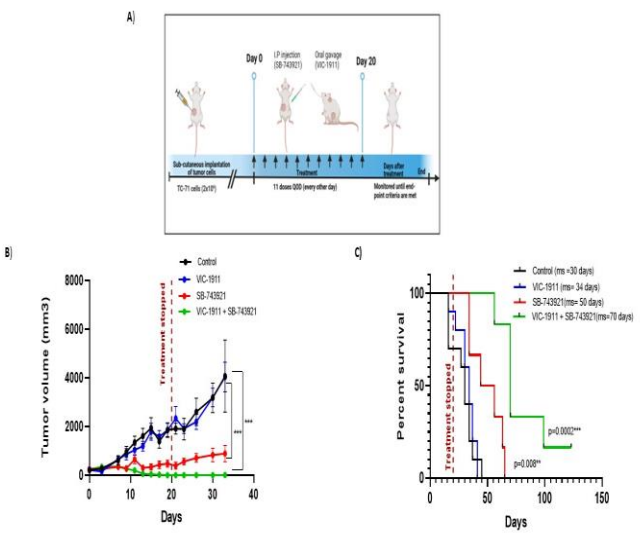
Dose-response curves, dose-response matrix, and heat map indicating synergy scores in different EWS cell lines. Combination treatment in resulted in the following synergy scores in A) TC-71 (24.62) B) SKES-1 (26.22), C) CHLA-258 (23.29), and D) COG-E-352 (30.13).

Figure 3. Combination treatment results in G2/M arrest, mitotic delay and apoptosis



Cell cycle analysis was performed on TC-71 cell line upon drug treatments and cells were assessed for changes in cell cycle profile 24 hours post treatment after Propidium Iodide staining. Different phases of cell cycles are represented for different groups including, **A**) control, SB-743921 (5 nM), VIC-1911 (10 nM) and combination treatment (SB-743921 2.5 nM + VIC-1911 10 nM). EWS cells treated with drugs were assessed for changes in protein expression of several proteins via capillary electrophoresis-based Wes analysis. **B**) Increased protein levels of KIF11, AURKA, cleaved PARP and KIF11 Thr926 phosphorylation was observed post-treatment.

Figure 4. Tumor regression with combination treatment in EWS xenografts



A) Timeline of in vivo study is represented.SB-743921 (2.5 mg/kg) and VIC-1911 (37.5 mg/kg) were dosed intraperitoneally and orally respectively. Mice were treated with these drugs every other day until 20 days and monitored for tumor progression and end-point symptoms. We observed significant B) tumor regression (control vs SB-743921, p<0.001*** and control vs combination, p<0.0001**** as assessed by one-way ANOVA), and C) overall survival with the combination treatment (VIC-1911 and SB-743921) compared to monotherapy and vehicle control groups (control vs SB-743921, p=0.008** and control vs combination, p=0.0002*** as measured by Log-rank (Mantel-Cox) test).

(P 447) TARGETING FOCAL ADHESION KINASE AUGMENTS THE THERAPEUTIC POTENTIAL OF BROMODOMAIN INHIBITORS IN EWING SARCOMA

Shireen S. Ganapathi, MD[1]; Nicolas M. Garcia, MS[1]; Aya Miyaki[1]; Yuqi Kang, MSc[2]; Marina Chan, PhD[2]; Taran Gujral, PhD[2]; Elizabeth R. Lawlor, MD, PhD[1]; Emma Wrenn, PhD[1], (1) Seattle Children's Research Institute, Seattle, WA, (2) Fred Hutchinson Cancer Center, Seattle, WA

Objective: Metastatic and relapsed Ewing sarcoma (EwS) portends a dismal prognosis and new therapeutic strategies are critically needed. EwS is driven by FET::ETS fusions, most commonly EWS::FLI1, which causes global transcriptional dysregulation via epigenetic reprogramming. Given the central role for epigenetic dysregulation in EwS, we are investigating the bromodomain inhibitor (BETi) BMS-986158, the first BETi to be tested in children and adolescents in an ongoing Phase 1 clinical trial (NCT03936465). To ensure translational success of BMS-986158, combination strategies must be identified. In this study we seek to identify biologically rational pathways that can be targeted in combination with BMS-986158 to enhance its therapeutic efficacy.

Methods: Whole-transcriptome bulk RNA-seq was performed on three EwS cell lines exposed to 5nM (IC50 dose) BMS-986158 for 72hrs, 20 days, or vehicle. A kinase inhibitor screen that exploits polypharmacy and uses a linear regression based mathematical model to identify potential kinase targets was performed in drug-tolerant cells that emerged after exposure to BETi for 20 days. 2D dose response and synergy assays assessed cytotoxicity of single agents and combinations respectively. Synergy was determined using both Chou-Talalay and Bliss Synergy models. Invasion in a physiologic 3D matrix was assessed using EwS spheroids plated on rat tail collagen and imaged using brightfield microscopy. Subcutaneous xenograft EwS

models in immunodeficient mice were used to test single agent and combinations identified as synergistic. Immunofluorescence and immunohistochemistry were used to detect protein expression in in vitro and in vivo specimens.

Results: Single agent BMS-986158 was initially cytostatic at low nanomolar IC50 but proliferation was restored by 20 days. In vivo treatment slowed xenograft tumor growth and increased survival (p-value 0.017, log-rank test), but tumors did not regress and eventually reach endpoint. Given outgrowth, in vitro drug-tolerant populations were investigated for mechanisms of escape. RNA-seq revealed global transcriptional rewiring with continued reversal of the EWS::FLI1 transcriptional signature, demonstrating on target effect despite restored proliferation. Interrogation of gene expression profiles showed that each cell line had acquired its own unique transcriptional signature, but pathway analysis of shared genes identified common programs. Top enriched terms among shared gene programs included: Hallmark-Myogenesis and EMT, Reactome- Collagen Formation, Integrin Cell Surface Interactions, and Gene Ontology- Cell Adhesion, Cell-Cell Signaling. This suggested that mesenchymal programs and integrin signaling had been reproducibly activated in BETi-tolerant cells. In parallel, a kinase inhibitor screen revealed focal adhesion kinase (FAK) to be one of four kinases that remained a dependency in the drug tolerant population. Phenotypically, drug tolerant cells had fibroblastic morphologies with increased F-actin filaments by phalloidin staining in 2D culture and significantly more invasive strands in 3D collagen assays compared to parent cells. Exposure of EwS cells to the FAK inhibitor Defactinib in combination with BMS-986158 showed that the agents were highly synergistic in both BETi-naïve and drug-tolerant populations. Cytotoxicity was enhanced in 2D, and in 3D spheroids cell viability and invasion were blocked across multiple EwS models. Combination studies are ongoing in EwS xenografts.

Conclusion: Our studies reveal that exposure of EwS cells to BETi leads to transcriptional rewiring that enables restoration of cell proliferation. Despite evidence of cell context-specificity, we observed that BET inhibition causes reproducible transition of EwS to more mesenchymal cell states which are dependent on FAK. This dependency can be therapeutically exploited by combining BETi with FAK inhibition, a combination which proved to be highly synergistic in vitro, with enhanced cytotoxic and anti-invasive properties. This work supports further investigation of FAK inhibitors as combination agents to enhance the efficacy of BETi, with the potential for translation to high-risk patients.

(P 448) CAF-LIKE EWING SARCOMA CELLS DEPOSIT PRO-TUMORIGENIC EXTRACELLULAR MATRIX IN RESPONSE TO TGF-BETA SIGNALING

Emma Wrenn, PhD[1]; April Apfelbaum, PhD[2]; Erin Rudzinski, MD[3]; Xuemei Deng, n/a[4]; Katherine Braun, PhD[1]; Nicolas M. Garcia, MS[1]; Trisha Lipson[5]; Aya Miyaki[1]; Virginia Hoglund, MS[1]; Shruti Bhise, n/a[6]; Sami Kanaan, PhD[6]; Olivia Waltner[6]; Scott Furlan, MD[6]; Elizabeth R. Lawlor, MD, PhD[1], (1) Seattle Children's Research Institute, Seattle, WA, (2) Dana Farber Cancer Institute, Boston, MA, (3) Seattle Children's Hospital/University of Washington, Seattle, WA, (4) Seattle Children's Hospital, Seattle, WA, (5) University of Washington, Seattle, WA, (6) Fred Hutchinson Cancer Center, Seattle, WA

Objective: Despite limited genetic and histologic heterogeneity, Ewing sarcoma (EwS) tumor cells are transcriptionally heterogeneous. Recently we identified subsets of highly mesenchymal EwS cells that deposit pro-tumorigenic extracellular matrix proteins. Due to their

role in ECM remodeling, which is typically carried out by cancer-associated fibroblasts (CAFs) in carcinomas, we termed them CAF-like cells. In this study we sought to further characterize the transcriptional heterogeneity and upstream drivers of these CAF-like states.

Methods: Single cell proteogenomic-sequencing (CITE-seq) of EwS cell lines was performed and integrated with patient tumor transcriptomic data. Tumor cell subpopulations were evaluated in cell line or patient-derived xenografts and patient tumor biopsies using multiplex immunofluorescence (mIF) staining. Digital spatial profiling and human whole transcriptome analysis interrogated spatial transcriptomic heterogeneity in EwS xenografts. Cell subpopulations were isolated by FACS for assessment of gene expression, proliferation, and migration. TGF-beta signaling was induced with recombinant TGFB1 and inhibited via CRISPR-Cas9 knockout of TGFBR2, lentiviral expression of a dominant negative TGFBR2 construct, or addition of small molecule TGFBR1/ALK4 inhibitor SB505124. Invasion was measured by invasive strand formation of tumor spheroids cultured in 3D collagen-I rich gels.

Results: CITE-seq of 9 EwS cell lines identified the cell surface protein CD73 (NT5E) as a biomarker of CAF-like EwS cells in vitro. CAF-like EwS cells deposited extracellular matrix (ECM) proteins including Tenascin-C (TNC), COL1A1, Biglycan, and SPARC in vitro and CD73+ cells co-localized with areas of ECM-deposition in xenografts and patient tumor samples in vivo. Gene ontology (GO) analysis of genes that were reproducibly upregulated in NT5E+ vs. NT5E- cells by single cell sequencing showed highly significant enrichment of GO terms “TGF-beta regulation of extracellular matrix” (NCATS BioPlanet, p.adj = 5.3E-11) and SMAD2 binding (ChEA, p.adj = 4.4E-14), suggesting that TGF-beta may regulate the CAF-like state. In support of this, flow cytometry of EwS cell lines revealed a 3-fold increase of TGFBR2 surface expression in CD73+ vs. CD73- cells. TGFB1 treatment of EwS cells in vitro induced expression of NT5E as well as CAF-like signature genes BGN, COL1A1, SPARC, and TNC (p. adj < 8.5E-5). In contrast, knockout of TGFBR2 or expression of a dominant negative TGFBR2 led to reduced expression of these genes. mIF of patient tumors and xenografts revealed the presence of subpopulations of phospho-SMAD2 S465/467+ tumor cells adjacent to areas of TNC deposition. Phenotypically, modulation of TGF-beta signaling had minimal impact on EwS cells grown in 2D culture, but TGF-beta inhibition reduced invasion and viability in 3D collagen I-rich culture.

Conclusion: EwS tumors generate subpopulations of tumor cells that deposit pro-tumorigenic ECM, functioning analogously to CAFs. EwS cells have been reported to be non-responsive to TGF-beta signaling due to EWS::FLI1-mediated repression of TGFBR2. Here, we report that CAF-like EwS cells express TGFBR2 and respond to TGF-beta ligands. We find that TGF-beta signaling is a master regulator of CAF-like states and ECM deposition in Ewing sarcoma. This raises the possibility of TGF-beta inhibition as a therapeutic strategy to inhibit tumor progression by targeting the tumor microenvironment.

(P 449) **CIC-DUX4 sarcomas: A single-center review of clinical features and molecular aberrations identified through DNA sequencing**

Victoria Wytiaz, MD[1]; Douglas Rottmann, MD[2]; Dafydd Thomas, MD, PhD[2]; Scott Schuetze, MD, PhD[3]; Christina V. Angeles, MD[4]; Rashmi Chugh, MD[5], (1) University of Michigan, Department of Hematology/Oncology, Ypsilanti, MI, (2) University of Michigan, Department of Pathology, Ann Arbor, MI, (3) University of Michigan, Department of Hematology/Oncology, Ann Arbor, MI, (4) Department of Surgery, Rogel Cancer Center, University of Michigan, Ann Arbor, MI, (5) University of Michigan Rogel Cancer Center, Ann Arbor, MI

Objective: CIC-rearranged sarcomas, a rare and distinct subtype of undifferentiated round cell sarcomas, harbor CIC-related gene fusions. While other fusion partners exist, a CIC-DUX4 fusion is present in 95% of cases. Despite a small blue round cell morphology, CIC-rearranged sarcomas appear more aggressive and less responsive to treatment with inferior outcomes when compared to Ewing sarcoma. Given the rarity of CIC-rearranged sarcomas, further investigation into these poorer outcomes is scarce, as are consensus treatment guidelines. The aim of this study is to summarize clinical and histological features of CIC-DUX4 sarcomas and to review additional molecular aberrations identified by DNA sequencing to facilitate targeted therapy.

Methods: A single-institution retrospective chart review using a free-text search engine and keywords “CIC-DUX4”, and “sarcoma” generated 25 patients of any age diagnosed from 2012-2022. Eligible patients had a confirmed diagnosis of CIC-DUX4 sarcoma based on immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), molecular profiling via next generation sequencing or the Michigan Oncology Sequencing Center (MI-ONCOSEQ), an integrative sequencing technique that detects point mutations, insertions/deletions, gene fusions and rearrangements, amplifications/deletions, and outlier expressed genes.

Results: 15 eligible patients (10M/5F) were identified with a median age of 23 years (range 10-85); 8 patients (53%) were 15-39 years at the time of diagnosis. 7 patients were non-white (5 black and 2 Hispanic). 8 patients (53%) presented with localized disease and 7 patients had advanced (metastatic or unresectable) disease at diagnosis. Sites of metastases included lung (n=7), adrenal (n=1), and bone (n=1). 10 patients (67%) received radiotherapy to the primary site, 6 as part of definitive treatment, 3 for palliation and 1 for pre-operative treatment. 9 patients (60%) underwent surgical resection of the primary site. 14 patients received chemotherapy, primarily Ewing sarcoma-based regimens, while one patient received pazopanib as primary therapy. 7 patients (47%) received additional lines of systemic therapy. 1-, 2-, and 5-year overall survival (OS) was 67%, 32%, and 7%, respectively. OS estimates for patients with localized disease were 76%, 39%, and 13% at 1, 2 and 5 years while patients presenting with metastases had OS estimates of 57%, 28% and 0% at these times points. 2 patients with metastatic disease and 1 patient with localized disease remain in follow-up without evidence of disease at 37, 45, and 85 months, respectively, following diagnosis. MI-ONCOSEQ in available specimens revealed aberrations in ETS family genes, cell cycle gene CCNE1, FGFR1 and FGF1 and others. Histology was reviewed in 13 cases to identify features to help distinguish these tumors from Ewing’s sarcoma. 13/13 showed necrosis, 10/13 (77%) had myxoid matrix, 10/13 (77%) showed cytoplasmic clearing and 2/13 (15%) had moderate nuclear pleomorphism. Spindle cell or rhabdoid features were noted in 6 cases (46%).

Conclusion: In this series, CIC-DUX4 sarcomas affected young adults although in lower incidence than in other series. Racial distribution differed from that seen with Ewing sarcoma, with a higher incidence in non-white persons. While overall prognosis with CIC-rearranged

sarcomas is poor, survival duration of more than 3 years occurred in a small subset of patients treated with multimodality therapy. Evaluating specific histological features of these CIC-DUX4 sarcomas to distinguish from Ewing's sarcoma can help guide a correct diagnosis when molecular testing is not readily available. DNA sequencing in our series revealed a non-homogenous pattern of outlier expressed genes which may inform targets for individualized therapy beyond standard Ewing sarcoma-based regimens.

(P 450) INVESTIGATING THE THERAPEUTIC POTENTIAL OF SMALL MOLECULE INHIBITOR INOBRODIB AS A TREATMENT ALTERNATIVE FOR CIC-DUX4 SARCOMA

MaKenna R. Browne, B.S.[1]; Peter G. Hendrickson, MD,PhD[2]; Kristianne M. Oristian, PhD[1]; Ian Lock, B.A[1]; David Kirsch, MD, PhD[3], (1) Duke University, Durham, NC, (2) Duke Univeristy, Durham, NC, (3) Princess Margaret Cancer Centre/University Health Network, Toronto, ON, Canada

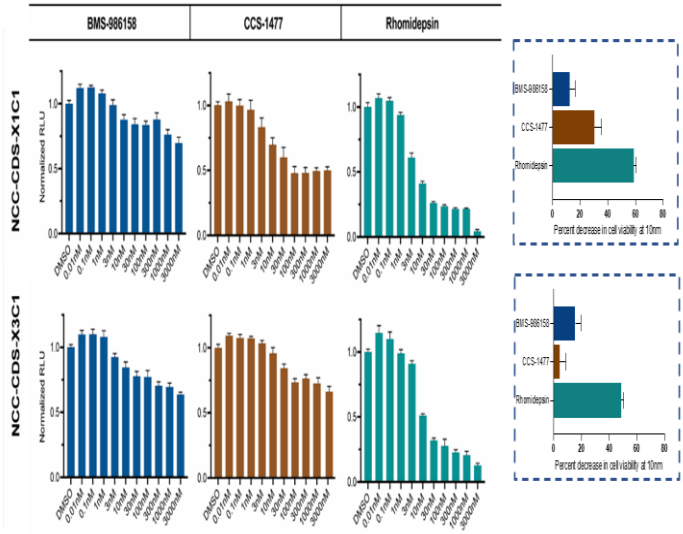
Objective: CIC-DUX4 Sarcoma (CDS) is a newly recognized subtype of undifferentiated round cell sarcoma driven by the fusion between cell cycle regulator and transcriptional repressor Capicua (CIC) and pioneer transcription factor DUX4. The CIC-DUX4 oncoprotein contains the N-terminal DNA binding domain of CIC and C-terminal transactivation domain of DUX4 which interacts with histone acetyltransferase complex P300/CBP to induce transcriptional activation. Previous studies have shown that iP300W- a selective P300/CBP histone acetyltransferase (HAT) domain inhibitor-induces cell cycle arrest and prevents growth of CDS xenografts. However, the clinical utility of iP300W remains a concern as bio-similar compounds such as A-485 have exhibited several dose-limiting toxicities in pre-clinical models. Here we investigated the therapeutic efficacy of clinically available alternative called CCS1477 (Inobrodib) which is a selective small molecule inhibitor of the P300/CBP bromodomain. Inobrodib is currently being evaluated in phase I/II clinical trials for prostate cancer and hematological malignancies with early results demonstrating a favorable therapeutic ratio.

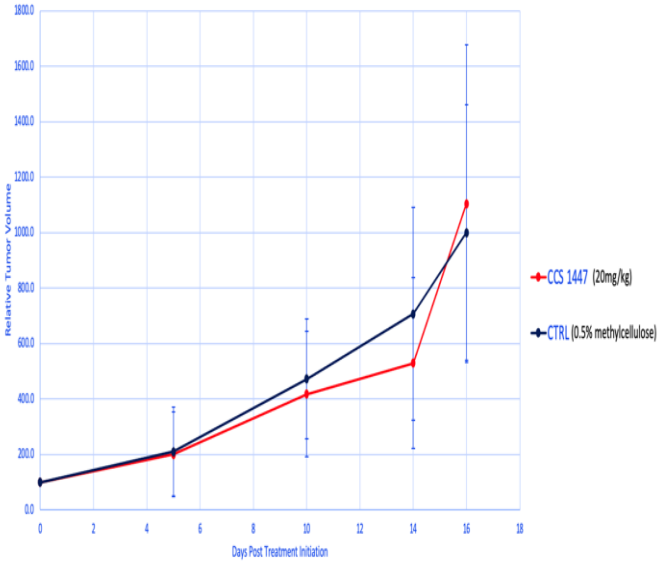
Methods: We modeled CDS by generating subcutaneous xenograft tumors in immunodeficient CIEA NOG mice. In total, 20 CIEA NOG mice were transplanted with CDS tumor derived NCC-CDS-X1C1 cells resuspended in a media/Matrigel mixture. After palpable tumor masses were detected at the site of injection, the animals were treated with CCS1477 (or vehicle control) once daily by oral gavage for 21 consecutive days. The drug dose (20mg/kg) was selected based on bioavailability data and maximum tolerability observed in previous pre-clinical studies. Tumor growth was measured weekly using digital calipers and the animals were monitored closely for adverse effects. At 21 days, or pre-determined humane endpoint, the animals were euthanized and tumors were collected for additional studies including immunohistochemistry and western blot. In parallel, CellTiter-Glo assays were used to assess CDS tumor cell viability in vitro following treatment with CCS1477, Romidepsin (histone deacetylase inhibitor; HDAC) and BMS986158 (bromodomain inhibitor).

Results: The P300/CBP bromodomain inhibitor, CCS1477, did not prevent growth of CIC-DUX4 Sarcoma xenografts. The relative tumor volume at completion of the study was used as criterion of efficacy. The average relative tumor volume of the CCS1477 group was not significantly different than the average relative tumor volume of the control group at day 16 post treatment initiation. Moreover, in our hands, drug treatment was associated with the development of a severe lung infection/abscess which occurred in 4/10 mice (40%) compared

to 0/10 in the control group. In vitro, treatment with CCS1477 resulted in only modest reduction in cell viability (~30% at 10nM). Unexpectedly, a control group treated with a selective class 1 HDAC inhibitor Romidepsin yielded the most significant reduction in CDS tumor cell viability. These results suggest that opposed to depleting acetylation with the use of P300/CBP inhibitors, hyperactivating the CDS transcriptional program by increasing histone acetylation could be a more effective therapeutic strategy in CDS.

Conclusion: P300/CBP has been identified as a potential therapeutic target for CDS. Previous data showed that small molecule inhibition of the of the P300/CBP HAT domain using iP300W induces cell cycle arrest and prevents growth of CIC-DUX4 Sarcoma xenografts. Conversely, our studies indicate that treatment with P300/CBP selective bromodomain inhibitor has no effect on tumor growth and is associated with high rates of toxicity in mice. Corollary in vitro studies suggest instead that HDAC inhibitors, which likely work by hyperactivating the CDS transcriptional program, could represent a more efficacious treatment alternative.





(P 451) IMPACT OF TIME TO DIAGNOSIS - A PROSPECTIVE ANALYSIS OF THE EWING 2008 TRIAL

Josephine Kersting, MD[1]; Andreas Ranft, N/A, Dr.[2]; Vivek A. Bhadri, MD, PhD[3]; Bénédicte Brichard, MD[4]; Stéphane Collaud, MD[5]; Sona Cyprovà, MD[6]; Hans Eich, MD[7]; Torben Ek, PhD, MD[8]; Hans Gelderblom, MD, PhD[9]; Jendrik Hardes, MD[2]; Lianne M. Haveman, MD, PhD[10]; Wolfgang Hartmann, N/A, MD[7]; Peter Hauser, PhD, MD[11]; Heribert Jürgens, MD[7]; Jukka Kanerva, MD, PhD[12]; Thomas Kühne, MD[13]; Anna Raciborska, MD[14]; Jelena Rascon, PhD, MD[15]; Arne Streitbürger, MD[2]; Beate Timmermann, MD[2]; Yasmin Uhlenbruch, MD[16]; Uta Dirksen, MD[17]; Wiebke K. Guder, MD[18]; Maximilian Kerkhoff, MSc[19], (1) Pediatrics III, University Hospital Essen, West German Cancer Center, Duesseldorf, Nordrhein-Westfalen, Germany, (2) University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (3) Chris O'Brien Lifehouse, Sydney, New South Wales, Australia, (4) Cliniques Universitaires Saint Luc, Brussels, Brussels Hoofdstedelijk Gewest, Belgium, (5) Cologne Merheim Hospital, Köln, Nordrhein-Westfalen, Germany, (6) Motol Children's Hospital, Prague, Hlavni mesto Praha, Czech Republic, (7) University Hospital Münster, Münster, Nordrhein-Westfalen, Germany, (8) University of Gothenburg, Gothenburg, Vastra Gotaland, Sweden, (9) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, (10) Princess Máxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands, (11) Semmelweis University, Budapest, Budapest, Hungary, (12) HUS Helsinki University Hospital, New Children’s Hospital, Finland, Helsinki, Uusimaa, Finland, (13) University Children's Hospital Basel, Basel, Basel-Stadt, Switzerland, (14) Insitute of Mother and Child, Warsow, Mazowieckie, Poland, (15) Vilnius University Hospital, Vilnius, Vilniaus Apskritis, Lithuania, (16) St. Josef's Hospital Bochum, Bochum, Nordrhein-Westfalen, Germany, (17) University Hospital Essen, Pediatrics III, West German Cancer Center, German Cancer Research Center (DKTK) site Essen, National Center for Tumordiseases (NCT) site Essen, ,, Essen, Nordrhein-Westfalen, Germany, (18) Department of

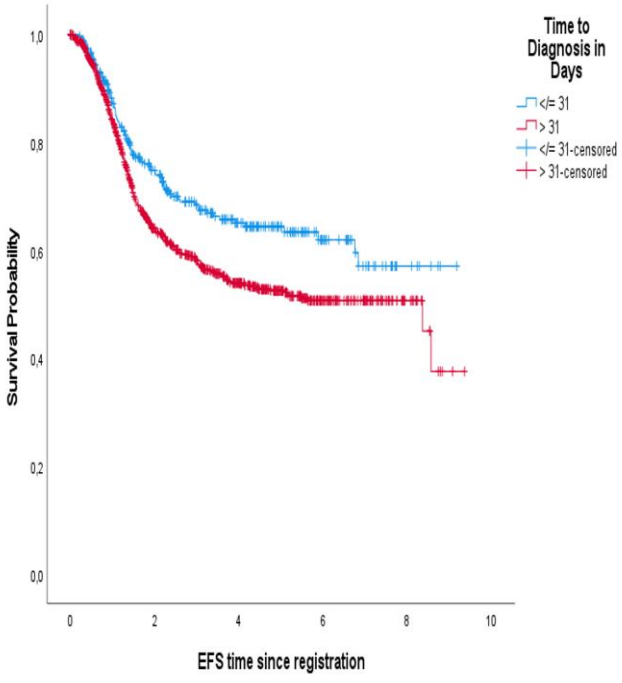
Orthopedic Oncology, University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (19) Pediatrics III, University Hospital Essen, West German Cancer Center, Essen, Nordrhein-Westfalen, Germany

Objective: Time to diagnosis (TtD) is defined as the time in days between the first symptom and the date of diagnosis. For Ewing Sarcoma (EwS) it has been reported to be amongst the longest in paediatric cancers. Results of previous studies seem to not show an association between time and outcome. Impact on time-to diagnosis on outcome was a secondary objective in the Ewing 2008 trial (NCT00987636).

Methods: A total of 1411 patients were enrolled into the Ewing 2008 trial and were therefore treated with the same treatment protocol. Information on patient-reported date and type of first symptom, including pain, pathological fracture and swelling, were prospectively gathered at time of diagnosis.

Results: TtD was calculated for 1265 patients. Median TtD was 82 days (IQR 31-180 days). TtD was longer for patients older than 15 years of age and pelvic tumours (p>.001). Univariable analysis revealed a HR 1.41 (p=.003) between time and EFS at a cut-off of 31 days. In localized and metastatic disease HR in univariable regression analysis were 1.22 (p=.22) and 1.48 (p=.02), respectively. Multivariable regression analysis revealed HR of 1.30 (p=.03) for time. Subgroup multivariable analysis for metastatic disease revealed HR=1.59 (p=.01) for time.

Conclusion: Results of this analysis demonstrate an association between longer TtD and outcome at a cut-off of 31 days. This effect was particularly strong in metastatic disease.



		95% CI		
	HR	Lower	Upper	*p-value*
Age (>15years)	1.42	1.20	1.73	*<.001*
Sex (female)	1.01	.83	1.23	*.91*
Primary Tumour Site (Pelvis)	1.17	.95	1.45	*.15*
Metastasis (yes)	3.35	2.73	4.10	*<.001*
Tumour Volume (≥200 ml)	1.39	1.13	1.70	*.002*
Registration (Study patient)	.80	.64	0.988	*.04*
Time to Diagnosis (>31 days)	1.30	1.02	1.66	*.03*

		95% CI		
	HR	Lower	Upper	*p-value*
Age (>15years)	1.21	.93	1.58	*.16*
Sex (female)	1.01	.78	1.31	*.94*
Primary Tumour Site (Pelvis)	.99	.75	1.30	*.93*
Tumour Volume (≥200 ml)	1.28	.98	1.68	*.07*
Registration (Study patient)	.82	.59	1.12	*.21*
Time to Diagnosis (>31 days)	1.59	1.12	2.27	*.01*

(P 452) SECONDARY GENOMIC LANDSCAPE OF CIC::DUX4 FUSION SARCOMAS

Nathan D. Seligson, PharmD[1]; Eman A. Gabal, BS[1]; Annemarie Rodriguez, BS[1]; James L. Chen, MD[2], (1) University of Florida, Jacksonville, FL, (2) The Ohio State University, Columbus, FL

Objective: CIC::DUX4 fusion sarcomas are a distinct subset of high-grade undifferentiated round cell sarcomas characterized by a specific oncogenic translocation involving the CIC and DUX4 genes mostly in pediatric population. These sarcomas are rare and aggressive, and there remains a lack of consensus in how CIC::DUX4 sarcomas should be treated clinically. Beyond the disease-defining fusion event, there is limited knowledge about the secondary genomic landscape associated with these sarcomas. We have previously reported that other small round cell tumors often have associated secondary alterations. In this study, we assess this hypothesis through evaluation of the genomic landscape of CIC::DUX4 sarcomas.

Methods: Genomic profiling data of clinical sarcoma specimens were collected from those analyzed by Foundation Medicine from 2012 through 2018 as originally approved by the Western institutional review board (IRB 20152817). Tumors harboring CIC::DUX4 (or DUX4 family members) fusions were selected. Only known pathogenic genomic variants were included. Pathway analysis for genes and targetable gene variants were defined by MSK OncoKB.

Results: Out of a total of 7,494 sarcomas, 16 patients with CIC::DUX4 fusions were identified. An addition two patients exhibited a CIC::DUX4L fusion and two patients exhibited a CIC::DUX4L4 fusion. Patients were primarily female (n=14, 70.0%%) with a median age at sequencing of 31 years (inter-quartile range: 17.25-50.25 years). Tumor mutational burden was low (median: 0.8 mut/Mb; inter-quartile range: 0-3.0 mut/Mb) and no tumors were microsatellite instable. Of the 20 total patients included, 10 (50.0%) did not exhibit any additional genomic variants. Of the 10 patients that did have secondary variants a total of 14 unique pathogenic genomic variants were identified. Only CCT6B was identified in two tumors. We next analyzed whether these variants shared common molecular pathways. Common pathways noted included DNA damage response (4/20), growth signaling (3/20), and transcription regulation (n=4/20). Three patients exhibited potentially targetable gene variants according to OncoKB including ATM insertion (olaparib), CHEK2 copy number deletion (olaparib), and FGFR3 splice site variant (erdafitinib).

Conclusion: Secondary genomic variants are rare in CIC::DUX4 sarcomas and show few molecular similarities. Potentially targetable genomic variants were also rare. This data suggest that the driving oncogenic signal for this malignancy may solely be a function of the CIC::DUX4 fusion. Further prospective research is needed to confirm these findings.

(P 453) ROLE OF SECOND-LINE TREATMENT IN EWING SARCOMAS WITH POOR PROGNOSIS AT DIAGNOSIS: EXPERIENCE OF A SPANISH REFERENCE CENTER.

Irene Carrasco Garcia, MD[1]; Julio Torres Sempere, n/a[2]; Gala Martinez Bernal, MD[2], (1) Virgen del Rocio University Hospital, Seville, Andalucia, Spain, (2) Hospital Universitary Virgen del Rocio, Seville, Andalucia, Spain

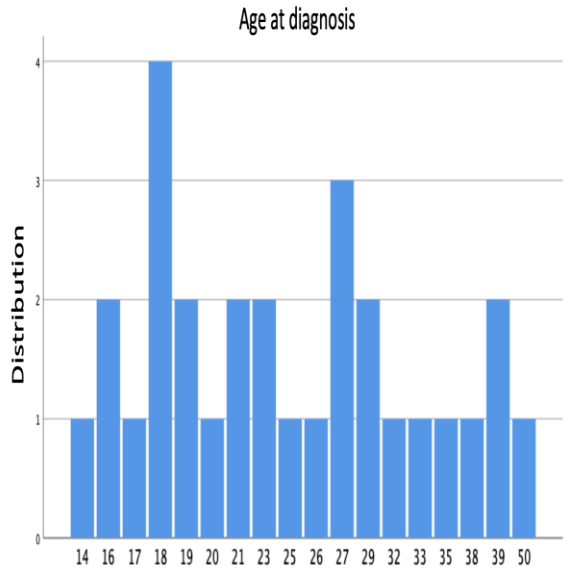
Objective: Relapsed Ewing sarcoma comprises an aggressive disease with a 5-year overall survival rate of about 15%. Prognosis is poor, especially if relapse occurs within the first 2 years after diagnosis. The REECur trial is the first to address this issue, with high-dose Ifosfamide being the best treatment in the second line, resulting in a progression free survival (PFS) of 5.7 months (m (95% CI,3.8-7.0) and a median overall survival (OS) 16.8 months (95% CI, 11.1-25.8).
The aim of our study was to describe our experience with second-line treatment in a reference center, focusing on patients with worse prognosis at diagnosis (high volume, metastatic at debur, or less than 90% necrosis after neoadjuvant treatment).

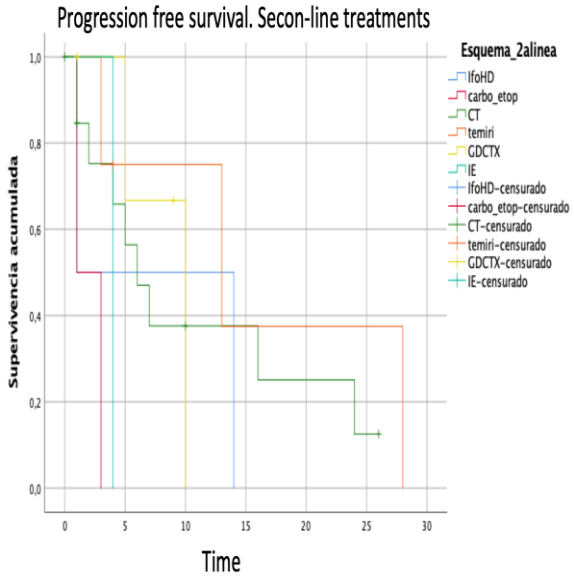
Methods: Patients diagnosed with Ewing sarcoma were treated with VAC/IE schema (regimen consisting of two parts: 7 cycles of VDC (vincristine, doxorubicin, cyclophosphamide) alternating with 7 cycles of IE (ifosfamide and etoposide)). At relapse, there were treated with different approved schemas.
We retrospectively analyzed 28 patients with poor prognosis at diagnosis who were with different schemas in the second line: cyclophosphamide + topotecan (CT) (14), high dose ifosfamide (IfoHD)(4), gemcitabine plus docetaxel (GemDoc)(4), irinotecan plus temozolamide (TEMIRI) (4), carboplatin and etoposide (CE) (2) and retreatment with Ifosfamide and etoposide (IE) (1)
Descriptive analysis was done, and the response to second-line treatment was analyzed based on three different factors: volume at debut, metastatic setting, and response to neoadjuvant treatment.

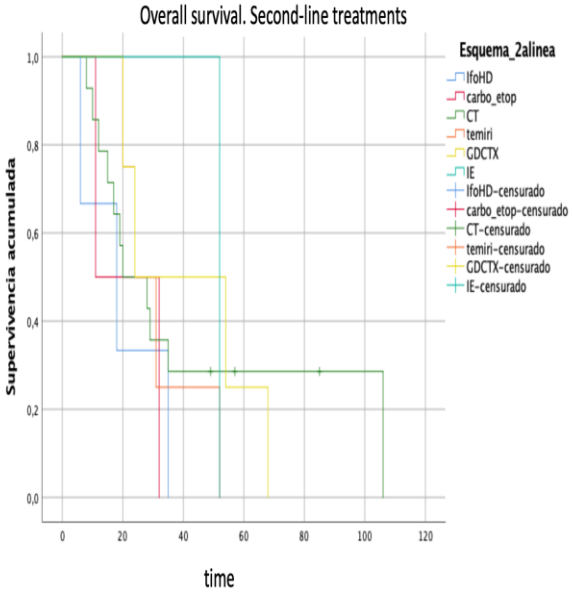
Results: Twenty-eight patients (pts) with relapsed Ewing sarcoma were analyzed retrospectively. The median age at diagnosis was 25 years (14-50) with a predominance of male patients (17 vs 11). 51,7% (15pts) presented with metastatic disease at debut (15), predominantly lung metastasis (41,1%). The median volume of the localized tumor was over 600cc. The time-lapse between first line and recurrence was 16 m.
The median PFS and OS for each schema were as follows: TEMIRI PFS 16 m (2-29) OS 31m (17-45), CT PFS 10,6 (5-16) OS44m (22-65), GemDoc PFS 8,3 (4-12) OS 41 (18-64), IfoHD PFS 7,5 (0-20) OS 19 (3-36) and CE PFS 2 (0-3) OS 21,5 (0-42). However, the differences were not significant (p=0,1)
In terms of metastatic debut, this patients had a shorter OS. However, the most effective schema was GemDoc (37m) followed by TEMIRI (35m). In patients with non-metastatic debut, CT showed better OS (60m).
In case of high volume at debut, again patients with a higher primary tumor volume had a worse prognosis, with an OS of 36 m (25-47) compared to 38m (22-54) in those with a lower volume. In this setting, CT showed better OS, than others (40m).
Analyzing the OS in patient who did not achieve necrosis over 90% (only surgical patients), CT showed the best OS (41m), and again, a difference was observed with a worse OS in this patients.

Conclusion: The debut of the disease is a prognostic factor in Ewing sarcoma: metastatic debut, high tumor volume, and necrosis under 90% after neoadjuvant treatment showed a

lower OS. The worst context is metastatic debut.
The REECur study is the first to analyze different second-line treatments; however, no differences were observed in tumors with poor prognosis.
In our retrospective study, TEMIRI had the best PFS (16m), but CT has better OS 44m in patients with a poor prognosis.
Analyzing the different settings in these patients, excluding metastatic disease CT showed the best data.







(P 454) TREATMENT PATTERNS AND OUTCOMES IN PEDIATRIC AND ADULT PATIENTS WITH EWING AND UNDIFFERENTIATED ROUND BLUE CELL SARCOMAS IN ALBERTA

Bilal Marwa, MBBS, MSc[1]; Jan-Willem Henning, MBChB, FRCPC[2]; Xiaolan Feng, MD PhD[2]; Mays Ibrahim, RD[2]; Jordan M. Stosky, MD, FRCPC, DABR[2]; Michael Monument, MD MSc FRCS(C)[3]; Melanie Finkbeiner, MD, FRCPC[4]; Omar Farooq Khan, MD, FRCPC[2], (1) Jim Pattison Children's Hospital, Saskatoon, SK, Saskatoon, SK, Canada, (2) Tom Baker Cancer Center, Calgary, AB, Canada, (3) University of Calgary, Calgary, AB, Canada, (4) Alberta Children's Hospital, Calgary, AB, Canada

Objective: Ewing sarcoma (ES) and undifferentiated round cell sarcomas (URCS) are aggressive tumors of bone and soft tissues affecting adolescents and young adults. Standard treatment consists of systemic treatment in addition to surgery and/or radiation therapy (RT) for locoregional management. Generally, surgery is accepted as the modality of choice whenever feasible without significant morbidity. However, the indications for RT either as definitive locoregional management of ES/URCS or as an adjunct to surgery remains controversial. There are variations in the systemic management of ES/URCS in adult compared to pediatric ES/URCS, though it is unclear if the locoregional treatment (LRT) approach also varies in adult compared to pediatric patients. Our study aimed to investigate the LRT patterns and outcomes for ES and URCS within a publicly funded provincial healthcare system in Canada.

Methods: A retrospective chart review and analysis of pediatric and adult patients with ES or URCS treated in Alberta (with a catchment area of approximately 4.5 million people) from 2008 to 2022 was performed. Patients were selected based on a confirmed diagnosis of ES, peripheral neuroectodermal tumor or URCS reviewed by experienced sarcoma pathologists. Desmoplastic round cell tumors were excluded. Patient demographics, pathologic

characteristics (including molecular pathology results if available), disease stage at diagnosis, and treatment strategies (including type, timing, and intent of locoregional therapy) were collected in our Integrated Sarcoma Research Program (iSARP) REDCap database. Descriptive statistics were utilized to assess demographics and treatment trends over time. Kaplan-Meier survival and Wilcoxon rank-sum analysis were used to compare clinical outcomes between adult and pediatric patients for event-free survival (EFS) and overall survival (OS), and between patients receiving different LRT.

Results: Eighty patients were treated between 2008 and 2022 with ES or URSC including 67 patients with ES, three patients with CIC-rearranged sarcoma, and 11 patients with non-specified URCS (Table 1). Metastatic disease was present in 36% of all adult patients and in 57% of pediatric patients. EFS in patients with metastatic disease was 8.8 months for adults versus 19.6 months for pediatric patients ($p = 0.02$). OS for patients with metastatic disease was 11.5 months for adults and 34.5 months for pediatric patients ($p = 0.021$). In patients with localized disease, median EFS for adults was 33.0 months, while median EFS for pediatric patients was not reached ($p = 0.221$). Median OS for adults with localized disease was 99.1 months, while median OS for pediatric patients was not reached ($p = 0.518$, Figure 1). Combination LRT for localized disease was utilized in 34% of adults compared to only 20% of pediatric patients. All patients with localized disease at diagnosis received LRT, but only 67% of patients with metastatic disease received any form of LRT. A numerically larger proportion of adults with metastatic disease received no LRT (56%) compared to children (15%) (Table 2). Concomitant chemotherapy with radiation was provided to 43% of adults receiving RT in the upfront setting compared to 95% of children. Median EFS for all patients (regardless of stage at diagnosis) treated with surgery alone was not reached, versus 76.2 months for patients treated with combined modality LRT and 22.0 months for patients treated with radiation alone. Patients receiving no LRT (3 children and 9 adults) had a median EFS of 6.6 months.

Conclusion: In our cohort, combined modality LRT was used more frequently in adults with localized disease. In addition, adults with metastatic disease were less likely than children to receive any form of LRT in metastatic setting. Despite fewer adults having metastatic disease at initial diagnosis, outcomes for adults were poorer than for pediatric patients. It is unclear whether differences in LRT used in adults compared to pediatric patients contribute to poorer outcomes in adults. More studies are needed to assess the differential efficacy of LRT in adult and pediatric patients with ES and URCS in both localized and metastatic settings. We are planning to expand our analysis through a collaborative national study combining the datasets from several academic sarcoma centers in Canada.

Table 1. Baseline characteristics and treatment approaches for ES and URCS patients in Alberta from 2008 – 2022.

	Number of Patients	Percentage of Patients
Sex		
Male	51	64%
Female	29	36%
Age		
Pediatric (< 18 years)	35	44%
Adult (≥ 18 years)	45	56%
Range (years)	3 – 81	
Disease Extent at Diagnosis		
Pediatric (< 18 years)		
Localized	15	43%
Metastatic	20	57%
Adult (≥ 18 years)		
Localized	29	64%
Metastatic	16	36%
Pathologic Diagnosis		
Ewing sarcoma (with molecular pathologic confirmation)	57	71%
Ewing sarcoma (histologic diagnosis only)	10	13%
Undifferentiated round cell sarcoma	10	13%
CIC-rearranged sarcoma	3	4%
Molecular Pathology		
EWSR1 rearrangement (partner unknown)	38	48%
EWSR1::FLI1 fusion	17	21%
EWSR1::ETV6	2	3%
CIC rearrangement (partner unknown)	3	4%
Locoregional Treatment Strategy Utilized		
Radiation therapy	28	35%
Surgery	20	25%
Combined modality (radiation + surgery)	18	23%
None	12	15%
Unknown[1]	2	3%
Tumour Location		
Extremity	43	54%
Non-Extremity	37	46%
Year of Diagnosis		
Prior to 2012	27	34%
2013 – 2017	31	39%
2018 Onwards	22	28%

Table 2. Locoregional Treatment Strategy Utilized by Age and Disease Extent at Diagnosis

		Radiation Only		Surgery Only		Combined Modality (Surgery + RT)		None	
		#	%	#	%	#	%	#	%
Localized	Adult[1]	7	24%	10	34%	10	34%	0	0%
	Pediatric	6	40%	6	40%	3	20%	0	0%
Metastatic	Adult	4	25%	3	19%	0	0%	9	56%
	Pediatric	11	55%	1	5%	5	25%	3	15%

[1]Proportions do not add up to 100% for adults with localized disease as two patients were lost to follow-up prior to locoregional management.

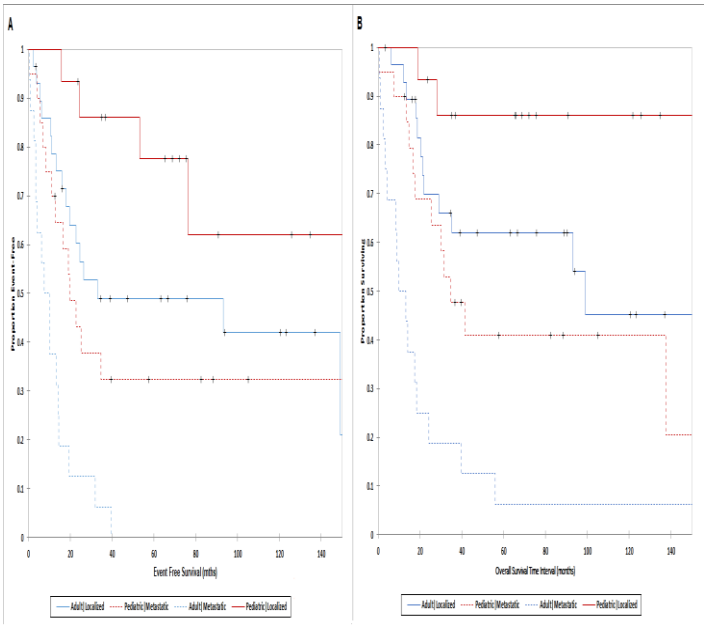


Figure 1: Event-Free Survival (A) and Overall Survival (B) for ES/URCS Patients in Alberta from 2008-2022 Stratified by Age and Stage at Diagnosis

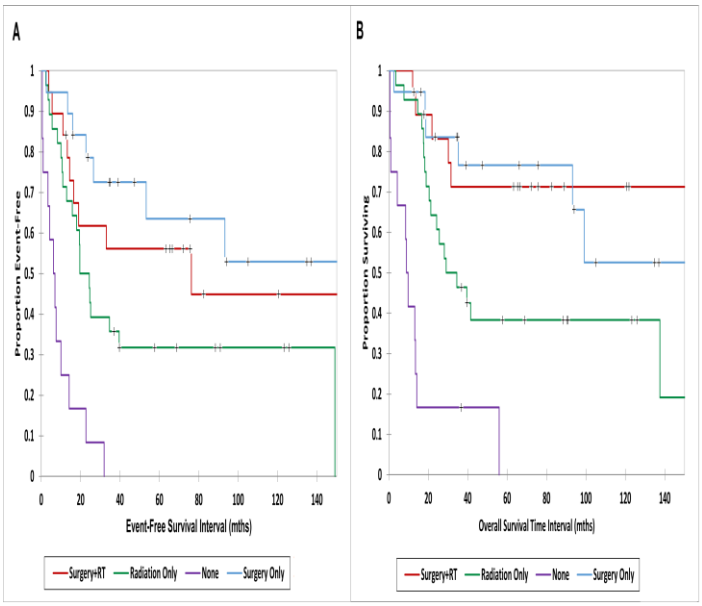


Figure 2: Event-Free Survival (A) and Overall Survival (B) for ES/URCS Patients in Alberta from 2008-2022 Stratified by Locoregional Treatment Modality

(P 455) EXTRASKELETAL EWING SARCOMA: A MULTICENTRIC RETROSPECTIVE STUDY FROM THE ITALIAN SARCOMA GROUP

Alessandra Borghi, MD[1]; Rosalba Miceli, MD, PhD[2]; Giacomo Giulio Baldi, MD[3]; Paola Collini, MD[4]; Salvatore Provenzano, MD[5]; Rossella Bertulli, MD[5]; Domenico Andrea A. Campanacci, Full Professor[6]; Davide Stimolo, MD[6]; Filippo Nozzoli, MD[7]; Giuseppe Bianchi, MD[8]; Marco Gambarotti, MD[9]; Anna Paioli, N/A, MD[10]; Antonella Brunello, MD, PhD[11]; Benedetta Chiusole, MD[12]; Marta Sbaraglia, MD[13]; Giovanni Grignani, MD[14]; Lorenzo D'Ambrosio, MD[15]; Alessandra Merlini, MD, PhD[16]; Paolo Giovanni G. Casali, MD[17]; Alessandro Gronchi, MD, FSSO[18]; Dario Callegaro, MD[1], (1) Sarcoma Service, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (2) Unit of Biostatistics for Clinical Research Department of Epidemiology and Data Science, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (3) Medical Oncology Department, "Santo Stefano" Hospital, Prato, Italy, Prato, Toscana, Italy, (4) Department of Advanced Diagnostics , Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (5) Mesenchymal and rare cancer Medical Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy, (6) Department of Orthopaedic Oncology and Reconstructive Surgery, Azienda Ospedaliero Universitaria Careggi, Firenze, Italy, Florence, Toscana, Italy, (7) Histopathology and Molecular Diagnostics, Careggi University Hospital Florence, Italy, Florence, Toscana, Italy, (8) Istituto Ortopedico Rizzoli IOR IRCCS, SC Third Orthopaedic Clinic and Traumatology, Bologna Italy, BOLOGNA, Emilia-Romagna, Italy, (9) Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (10) Istituto Ortopedico Rizzoli, Bologna, Emilia-Romagna, Italy, (11) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy, Padua, Veneto, Italy, (12) Oncology 1 Unit, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Veneto, Italy, (13) University of Padua, Padova, Veneto, Italy, (14) Candiolo Cancer Institute, FPO – IRCCS, Turin, Piemonte, Italy, (15) University if Turin, Turin, Italy, Torino, Piemonte, Italy, (16) Department of Oncology, University of Turin, Torino, Piemonte, Italy, (17) Department of Medical Oncology 2, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, Milan, Lombardia, Italy, (18) Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, Milan, Lombardia, Italy

Objective: Ewing sarcomas are aggressive mesenchymal tumors arising mostly in bones but, in about 20% of patients, also in superficial or deep soft tissues (Extraskeletal Ewing Sarcoma, EES). The aim of this retrospective multicenter study was to assess clinical behavior and oncologic outcomes of patients with primary EES treated within the Italian Sarcoma Group (ISG) network in light of the new WHO classification of small round cell sarcomas.

Methods: All consecutive patients of any age with primary localized (non-recurrent, non-metastatic) EES treated with surgery between January 2002 and December 2021 at one of the following ISG sarcoma reference centers were included: Fondazione IRCCS Istituto Nazionale dei Tumori, Milan; Azienda Ospedaliera Universitaria Careggi, Florence; IRCCS Istituto Ortopedico Rizzoli, Bologna; Istituto Oncologico Veneto - IRCCS, Padua; Istituto di Candiolo IRCCS, Department of Oncology at the University of Turin, Turin; Ospedale di Prato, Prato. Patients with a primary or secondary involvement of the bone were excluded.
All diagnoses were performed by pathologists with expertise in soft tissue tumors. The study included both patients with ES with known translocation partners (EWSR1-FLI1; EWSR1-ERG; EWSR1-ETS partners; FUS-ETS partners) and patients with EWSR1 translocations and unknown partners (not possible to assess, e.g exhaustion of the sample), where morphology and immunohistochemistry were consistent with ES.

Primary study endpoints were overall survival (OS), and crude cumulative incidence of local recurrence or distant metastases (CCI of LR or DM). Univariate and multivariable analyses were performed to investigate the association between prognostic variables and the study endpoints.

Results: The study included 89 patients, 71 (79.8%) adult, 18 (20.2%) pediatric (Table 1). Eighty-eight patients received chemotherapy (CT); of these, 17 (19.1%) received neoadjuvant CT, 60 (67.4%) neoadjuvant and adjuvant CT. Most common regimens included IVA-IE (19.5%), IVADo-IE (19.5%), and IVA (18.2%). One patient with a superficial 1cm EES was treated with surgery only.
After a median FU of 82.8 months (IQR 34.6-115.9), 5- and 8-yr OS were 0.80 (95%CI 0.71-0.90) and 0.78 (95%CI 0.69-0.88). At univariate analysis, patient's sex, age, radiotherapy (RT) administration, time period (2002-2011 vs 2012-2021) and tumor site, depth, size, and translocation type were not associated with OS (Table 2).
Five- and 8-yr CCI of LR were 0.08 (95%CI 0.04-0.17) and 0.11 (95%CI 0.05-0.21). LR occurred as first event in 8 patients: 7 deep EES and 1 superficial EES.
Five- and 8-yr CCI of DM were 0.24 (95%CI 0.16-0.36) and 0.28 (95%CI 0.19-0.42). At univariate analysis (Table 2), patient's age and tumor depth were associated with DM. Five-year CCI of DM was 0.61 (95%CI 0.38-0.99) in the pediatric group and 0.15 (95%CI 0.09-0.28) in the adult group (p 0.002, Fig.1A) while 5-yr CCI of DM was 0.00 in superficial and 0.31 (95%CI 0.21-0.46) in deep tumors (p 0.003, Fig.1B). Smaller tumors were associated with lower CCI of DM (p 0.074, Fig.1C). Unfavorable prognosis in pediatric group was confirmed at multivariable analysis (Fine and Gray model), after adjustment for size, depth, translocation type and RT administration (HR 2.84; 95%CI 1.19-6.78; p 0.019).
To further explore prognostic subgroups, patients were stratified by tumor size and depth. Five-year CCI of DM was 0.00 in 1-5 cm superficial EES (Group 1), 0.18 (95%CI 0.05-0.69) in 1-5 cm deep EES (Group 2), and 0.29 (95%CI 0.19-0.45) in deep EES >5cm (Group 3), p 0.054 (Fig.2A). There was a trend towards lower OS from Group 1 to Group 3 (Fig.2B, p 0.100).

Conclusion: Patients with superficial EES have a lower risk of developing DM, without a significant difference in OS. Tumor depth and size should be considered to stratify patients with EES and possibly identify favorable prognostic subgroups that might be offered less intensive medical treatments.

Figure 1. Five-year CCI of DM in the study population, considering age groups (adult population, pediatric population) (A), tumor depth (B), tumor size (C).

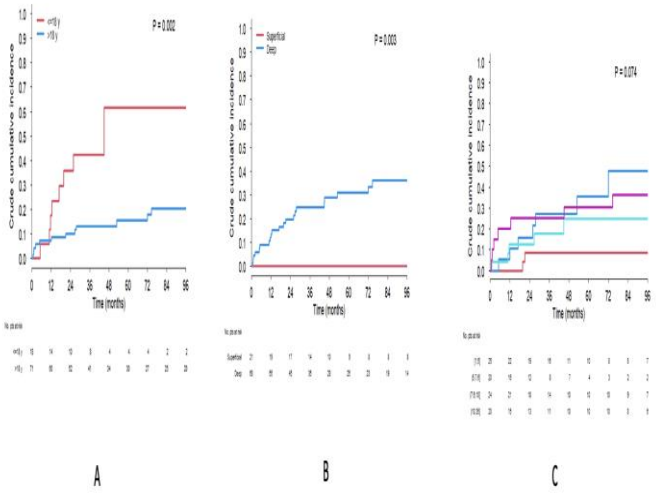


Figure 2. Five-year CCI of DM (A) and 5-year OS (B) considering combination of tumor depth and size: 1-5 cm superficial tumors (Group 1), 1-5 cm deep tumors (Group 2), >5 cm deep tumors (Group 3).

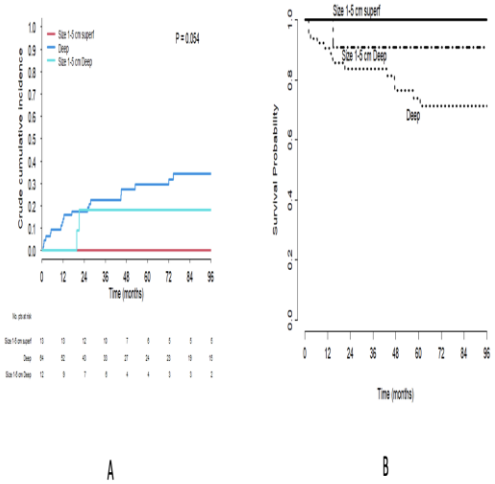


TABLE 1. Demographic, Clinical, and Molecular Characteristics of study population, adult population (>18 years) and pediatric population (≤ 18 years).

	Study population N = 89 n (%)	Adult population N=71 n (%)	Pediatric population N=18 n (%)	p*
Sex				0.789
Female	32 (36.0)	25 (35.2)	7 (38.9)	
Male	57 (64.0)	46 (64.8)	11 (61.1)	
Patients' age, y				-
Median	31	36	13	
(Q1 - Q3)	(21 - 45)	(26.5 – 48.5)	(10.5 – 6.75)	
Gene fusion				0.106 [a]
EWSR1-FLI1	49 (55.1)	35 (49.3)	14 (77.8)	
EWSR1-ERG	3 (3.4)	3 (4.2)	-	
FUS-ETS partner	1 (1.1)	1 (1.4)	-	
EWSR1-partner unknown	36 (40.4)	32 (45.1)	4 (22.2)	
Tumor size, cm				0.992
Median	7.6	7.0	8.0	
(Q1 - Q3)	(5.0 – 10.0)	(5.0 – 10.0)	(6.2 – 9.75)	
Depth				0.221
Superficial	21 (23.6)	19 (26.8)	2 (11.)	
Deep	68 (76.4)	52 (73.2)	16 (88.9)	
Type of surgery				0.177
Primary resection at reference center	72 (80.9)	55 (77.5)	17 (94.4)	
Re-excision	17 (19.1)	16 (22.5)	1 (5.6)	
Surgical margins				0.506
R0	68 (76.4)	53 (74.6)	15 (83.2)	
R1	12 (13.5)	11 (15.5)	1 (5.6)	
R2	6 (6.7)	5 (7.1)	1 (5.6)	
Not available	3 (3.4)	2 (2.8)	1 (5.6)	
Chemotherapy				-
Done (neoadj/adj/neo- and adjuvant)	88 (98.9) (17/11/60)	70 (98.6) (15/9/46)	18 (100.0) (2/2/14)	
Not done	1 (1.1)	1 (1.4)	-	
Radiotherapy				0.302
Done (pre/post/pre and postoperative)	45 (50.6) (15/29/1)	38 (53.5) (12/25/1)	7 (38.9) (3/4/-)	
Not done	44 (49.4)	33 (46.5)	11 (61.1)	
Clavien-Dindo ≥ 3a complications				0.438
y	11 (12.4)	8 (11.3)	3 (16.7)	
n	77 (86.5)	63 (88.7)	14 (77.7)	
Not available	1 (1.1)	-	1 (5.6)	

* P value at Fisher-Freeman-Halton exact test or Wilcoxon-Mann-Whitney test, where appropriate.
a: comparison EWSR1-partner unknown vs others.

Table 2. Univariable analyses: clinical-molecular variables predictive of 5-year OS and 5-year CCI of DM.

Variables n (%)	5-year OS (CI 95%)	p	5-year CCI of DM (CI 95%)	p
Sex		0.5		0.824
F: 32 (36.0)	0.861 (0.74 - 0.99)		0.24 (0.12 - 0.47)	
M: 57 (54.0)	0.761 (0.64 - 0.90)		0.24 (0.14 - 0.40)	
Age		0.3		0.002
≤ 18: 18 (20.2)	0.77 (0.56 - 1.00)		0.61 (0.38 - 0.99)	
>18: 71 (79.8)	0.80 (0.71 - 0.91)		0.15 (0.09 - 0.28)	
Tumor site		0.7		0.822
Retroperitoneum/pelvis: 15 (16.9)	0.85 (0.68 - 1.00)		0.21 (0.07 - 0.59)	
Viscera: 6 (6.7)	1.0 (1.00 - 1.00)		0.17 (0.02 - 0.23)	
Extremities: 49 (55.1)	0.79 (0.67 - 0.94)		0.29 (0.17 - 0.48)	
Trunk/chest wall/abdominal wall/head and neck: 19 (21.3)	0.71 (0.52 - 0.97)		0.18 (0.06 - 0.52)	
Tumor depth		0.2		0.003
Superficial: 21 (23.6)	0.90 (0.79 - 1.00)		0.00	
Deep: 68 (76.4)	0.77 (0.66 - 0.89)		0.31 (0.21 - 0.46)	
Tumor size		0.3		0.074
≤ 5.0 cm: 25 (28.1)	0.96 (0.88 - 1.00)		0.09 (0.02 - 0.34)	
5.0 - 7.6 cm: 20 (22.5)	0.69 (0.45 - 1.00)		0.35 (0.18 - 0.71)	
7.6 - 10.0 cm: 24 (26.9)	0.79 (0.61 - 1.00)		0.25 (0.11 - 0.56)	
10.0 - 35.0 cm: 20 (22.5)	0.68 (0.50 - 0.93)		0.30 (0.15 - 0.61)	
Tumor depth and size combination		0.1		0.054
Superficial, 1-5 cm: 13 (14.6)	1.00 (1.00 - 1.00)		0.00	
Deep, 1-5 cm: 12 (13.5)	0.91 (0.75 - 1.00)		0.18 (0.05 - 0.69)	
Deep: 64 (71.9)	0.74 (0.63 - 0.87)		0.29 (0.19 - 0.45)	
Time period		0.6		0.820
2002-2011: 33 (37.1)	0.84 (0.72 - 0.98)		0.22 (0.11 - 0.44)	
2012-2021: 56 (62.9)	0.77 (0.64 - 0.91)		0.26 (0.15 - 0.44)	
Radiotherapy		0.4		0.965
Yes: 45 (50.6)	0.86 (0.75 - 0.97)		0.24 (0.13 - 0.41)	
No: 44 (49.4)	0.73 (0.59 - 0.91)		0.25 (0.14 - 0.47)	
Gene Fusion		0.3		0.165
EWSR1- known partners: 52 (58.4)	0.78 (0.66 - 0.92)		0.31 (0.19 - 0.49)	
EWSR1 - unknown partners: 36 (40.4)	0.81 (0.69 - 0.96)		0.15 (0.07 - 0.35)	
Residual viable tumor		0.1		0.229
≥ 5%: 22 (24.7)	0.95 (0.87 - 1.00)		0.14 (0.02 - 0.10)	
<5%: 13 (14.6)	0.76 (0.51 - 1.00)		0.14 (0.05 - 0.40)	
Margin status		1.0		0.211
R0: 68 (76.4)	0.78 (0.69 - 0.92)		0.22 (0.13 - 0.36)	
R1/R2: 18 (20.2)	0.75 (0.57 - 1.00)		0.28 (0.13 - 0.60)	

(P 456) SECLIDEMSTAT TREATMENT BLOCKS THE TRANSCRIPTIONAL FUNCTION OF MULTIPLE FET-FUSION PROTEINS

Emily R. Theisen, PhD[1]; Galen Rask, M.S.[2]; Matthew Cannon, PhD[2]; Aundrietta Duncan, PhD[3]; John Sherman, PhD[2], (1) Nationwide Childrens Hospital, Columbus, OH, (2) Nationwide Children's Hospital, Columbus, OH, (3) Salarius Pharmaceuticals, Houston, TX

Objective: FET-rearranged sarcomas are characterized by chromosomal translocations involving the genes encoding a protein from the FET family (FUS, EWSR1, and TAF15). The fusion partner in these malignancies is most often a transcription factor and the resulting chimeric proteins function as oncogenic transcription factors. These fusion proteins recruit chromatin and transcriptional regulators to change the expression of thousands of genes. The protein domains included in the oncogenic fusions, including both low complexity domains and sequence-specific DNA binding domains, are difficult to pharmacologically target. Instead, efforts have been made to target critical co-regulators of the FET fusion proteins. The histone lysine specific demethylase 1 (LSD1) has been identified as a critical chromatin coregulator and inhibition with the noncompetitive inhibitor, SP-2509, has been shown to block the transcriptional function of EWS::FLI and EWS::ERG. The clinical analog of SP-2509, SP-2577 (seclidemstat) is currently in clinical trials for Ewing sarcoma. Multiple lines of evidence suggest LSD1 interacts with the FET domain of the fusion proteins and we wanted to evaluate whether seclidemstat treatment blocked transcriptional function of other known FET fusion proteins, including EWS::WT1, EWS::ATF, and FUS::DDIT3.

Methods: We used multiple cell lines to evaluate the transcriptional effects of seclidemstat including A673 (EWS::FLI), TTC-466 (EWS::ERG), JN-DSRCT-1 (EWS::WT1), BER (EWS::WT1), SU-CCS-1 (EWS::ATF), DTC1 (EWS::ATF), 402-91 (FUS::DDIT3), 1795-92 (FUS::DDIT3), DL221 (FUS::DDIT3). Dose response curves were generated after 96 hours of treatment for each cell line using Cell Titer Glo. Cells were treated with either the vehicle (0.3% DMSO), the IC50 for the cell line, or the IC90 for the cell line. Samples were collected for bulk RNA-sequencing at 24 hours and 48 hours. Sequencing was performed using standard approaches at the Institute for Genomic Medicine at Nationwide Children’s Hospital, and differential gene expression was determined and analyzed using previously published pipelines.

Results: We found that similar to SP-2509, seclidemstat causes widespread transcriptional changes in Ewing sarcoma cells and reverses both EWS::FLI and EWS::ERG function, in vitro. Having validated these findings, we next defined the fusion-driven transcriptional signature for each of the fusions and compared seclidemstat treatment to the fusion activity. We found that seclidemstat treatment also reverses the transcriptional function of the other FET-rearranged proteins, including both activated and repressed gene signatures. Additionally, we noted a limited change in gene expression at 24 hours, with extensive transcriptional reprogramming at 48 hours. Both the IC90 and IC50 doses induced changes in the same sets of genes. A small set of genes was commonly regulated across all of the cell lines.

Conclusion: These data indicate that seclidemstat blocks the transcriptional activity of FET-rearranged proteins in tumors other than Ewing sarcoma. This suggests LSD1 is critical co-regulator in other FET-rearranged malignancies. Our data also identify the heterogeneity in fusion-driven transcription and the response to seclidemstat, both across tumor types and with different cell lines of the same tumor type. This supports the highly context dependent role of the FET fusion proteins, as well as LSD1. Nonetheless, the commonly regulated genes suggest a key regulatory role for proteotoxic stress in the response to seclidemstat, providing a possible

pathway for combination strategies.

Orthopedic & Reconstructive Surgery

(P 457) LONG-TERM FOLLOW-UP OF PATIENTS WITH ATYPICAL LIPOMATOUS TUMOR (ALT) TREATED WITH MARGINAL RESECTION IN A REFERRAL CENTER

Laura Samà, MD[1]; Eva Covello, Student[1]; Laura Ruspi, MD[1]; Federico Sicoli, MD[1]; Vittoria D'Amato, Pharmacy[1]; Alice Laffi, MD[1]; Salvatore Lorenzo Renne, MD[1]; Davide Baldaccini, MD[1]; Elena Clerici, MD[2]; Alexia Francesca Bertuzzi, MD[3]; Pierina Navarria, MD[1]; Vittorio Lorenzo Quagliuolo, MD[1]; Ferdinando Carlo Maria Cananzi, MD[1], (1) IRCCS Humanitas Research Hospital, Rozzano, Milan, Lombardia, Italy, (2) Rozzano, Milan IRCCS Humanitas Research Hospital, Rozzano, Milan, Lombardia, Italy, (3) Humanitas Research Hospital, Rozzano, Lombardia, Italy

Objective: Atypical Lipomatous Tumor(ALT) of the extremities and superficial trunk, is a rare soft tissue tumor. The main treatment is marginal resection(MR). ALT have a propensity for to late local recurrence(LR) but unusually metastasize distantly. Our objective was to gain further insights into the timing of LR through the analysis of a cohort with a long follow-up (FU).

Methods: Data from 205 patients who underwent MR for ALT of the extremities or trunk at IRCCS Humanitas Research Hospital (Jan 1998-Jan 2023), were analyzed. Patients already published in 2014 were included, excluding 10 patients(9 spermatic cord liposarcoma,1 lost on FU). All patients had at least 10 years of FU. We included primary, recurrent tumors, and patients who underwent unplanned surgery(US) at another center, selected for re-excision. All patients were treated with MR, removing the tumor en bloc while preserving a slight cuff of muscle and/or surrounding soft tissues. Tumors were subclassified as lipoma-like or sclerosing subtypes. Tumor grade was assessed according to the Federation Nationale des Centres de Lutte Contre le Cancer (FNCLCC) criteria. The Clavien-Dindo Classification (CDC) was used for complications. After surgery, FU comprised physical examination and contrast-enhanced magnetic resonance imaging or ultrasonography in selected cases, every 6 months for 5 years and once a year thereafter. The primary outcome was local recurrence free survival (LRFS); overall survival (OS) was also calculated.

Results: A total of 111 patients were analyzed (Table 1). All ALT were FCNLCC Grade 1. Postoperative complications (CDC 1-2) were observed in 15 cases (13.5%). Median FU was 170.5months (IQR 132-199) for alive patients, and 134months (IQR 75-170) for dead patients. Median OS was not reached. Twenty-five patients(22.5%) died for ALT-unrelated causes. The 5-,10-,and 20-years OS rates were 97.3%,88.8%,69.2%, respectively. Median LRFS was not reached for either primary or recurrent tumors. At the last FU, 7 patients (6.3%) experienced LR. The 5-,10-,and 20-years LRFS rates for primary tumors were 94.7%,94.7%,94.7%, respectively. The 5-,10-,and 20-years LRFS rates for recurrent tumors were 100.0%,100.0%,69.2%, respectively (Figure 1). Among 98 patients with primary tumors, 5(5.1%) had recurrence. Median time to recurrence was 42months (IQR 12-44). Among 13 patients with recurrent tumors at presentation, 2(15.4%) had recurrence. Median time of recurrence 210.5months (IQR 202-219). In general, no additional recurrence was observed during the FU period after the initial recurrence. Characteristics of the 7 patients who

experienced a recurrence after surgery at our center are described in table 2. After diagnosis of recurrence, 1/7 patient (14.3%) did not receive any treatment, because of the clinical condition. However, 2/7 recurred patients (28.6%) underwent immediate surgical intervention. In the remaining 4/7 cases (57.1%), a wait and see approach was adopted. In these cases, surgical management was pursued upon evidence of dimensional progression of disease.

Conclusion: In our study, ALTs of the extremities and superficial trunk exhibited a LR rate around 6%. Through a long FU period, we observed a wide range of LR time (from few months to nearly 20 years). LR can be successfully managed with surgery. However, considering the rarity of secondary dedifferentiation and metastasis in ALT, a wait and see approach can be considered. Interestingly, we noted that patients who experienced late LR were initially treated at another center. According to our findings, it could be expected that after treatment for primary tumor, a LR may occur within the first 5 years. However, for patients who experienced a recurrence following an inadequate initial surgery, a late LR could be expected, potentially manifesting even up to 20 years later. This highlights the importance of long-term surveillance to ensure early detection and appropriate intervention.

Table 1: Patients' and tumors' characteristics

	Primary n=98	Recurrence n=13	Total N=111
Age, median (IQR)	58 (49-68)	62 (58-71)	59 (49-70)
Sex			
- M	64 (65.3)	6 (46.2%)	70 (63.1)
- F	34 (34.7)	7 (53.8%)	41 (36.9)
Size, median (IQR)	10 (5-17)	13.5 (6-20)	10 (5-18)
Site			
- Lower limb	53 (54.1%)	7 (53.8%)	60 (54.1%)
- Upper limb	12 (12.2%)	1 (7.7%)	13 (11.7%)
- Trunk	33 (33.7%)	5 (38.5%)	38 (34.2%)
Location			
- Superficial	47 (47.9%)	7 (53.8%)	54 (48.6%)
- Deep	51 (52.1%)	6 (46.2%)	57 (51.4%)
Histologic subtype (sclerosing)	10 (9.0%)	0 (0%)	10 (9.0%)
Tumor rupture	1 (0.9%)	0 (0%)	1 (0.9%)
Complications	12 (12.2%)	3 (23.1%)	15 (13.5%)
- CDC <3	12 (12.2%)	3 (23.1%)	15 (13.5%)
- CDC >=3	0 (0%)	0 (0%)	0 (0%)

IQR: interquartile rate
CDC: Clavien-Dindo Classification

Figure 1: Local recurrence free survival curve. A: All patients (N=111). B: Two groups, according to presentation (primary n=98, versus recurrence n=13).

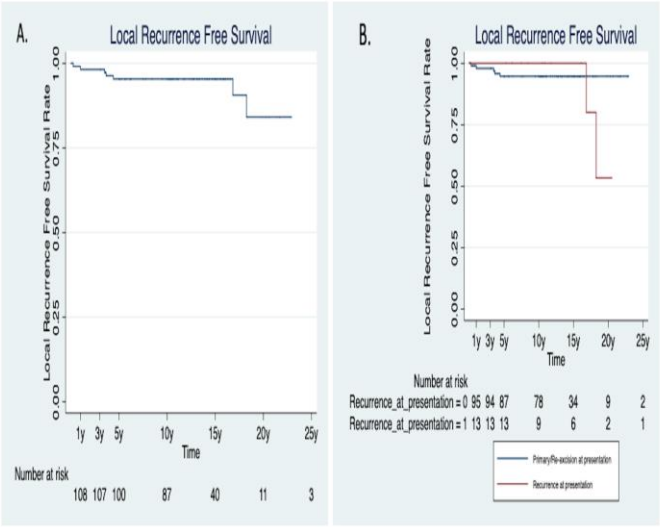


Table 2. Characteristics of patients who experienced a recurrence after surgery at our center

Patient	Age at diagnosis	Sex	Presentation at our Center	Size (cm)	Site	Location	Sclerosing subtype	Tumor Rupture	LRFS	Treatment of recurrence	Status
1	58	M	Recurrence	3.5	Trunk	Superficial	No	No	202	Surgery	NED
2	62	F	Recurrence	35	Lower limb	Deep	No	No	219	none	AWD
3	28	F	Primary	10	Trunk	Superficial	Yes	No	53	Surgery	NED
4	66	F	Primary	17	Lower limb	Deep	No	No	44	Wait and see. Surgery after 7 y	NED
5	66	F	Primary	12	Lower limb	Deep	Yes	No	3	Wait and see. Surgery after 6 y	NED
6	58	M	Primary	13.5	Trunk	Superficial	No	No	42	Wait and see. Surgery after 7 y	NED
7	72	M	Primary	5.5	Lower limb	Deep	No	No	12	Wait and see. Surgery after 8 y	NED

LRFS: local recurrence free survival
NED: non evident disease
AWD: alive with disease

(P 458) FREQUENCY IN CHANGE OF GRADING UPON LOCAL RECURRENCE OR DISTANT METASTASIS OF SOFT TISSUE SARCOMAS.

Maria Anna Smolle, PD MD[1]; Bernd Golja, cand.med.[2]; Dimosthenis Andreou, MD[3]; Bernadette Liegl-Atzwanger, Prof. MD[3]; Joanna Szkandera, MD[3]; Susanne Scheipl, PD MD[2]; Andreas Leithner, Prof. MD[3], (1) Department of Orthopaedics and Trauma, Medical University of Graz, Austria, Graz, Steiermark, Austria, (2) Department of Orthopaedics and Trauma, Medical University of Graz, Graz, Steiermark, Austria, (3) Medical University of Graz, Graz, Steiermark, Austria

Objective: The rate of local recurrences (LRs) and distant metastases (DM) in soft tissue sarcoma (STS) patients amounts to 10% and 30%, respectively. There is currently little evidence on the 1) frequency and 2) prognostic impact of change in grading upon development of LR or DM. The current retrospective single-centre study aimed at investigating the frequency of upgrading in case of LR or DM in STS, as well as the impact of upgrading on post-recurrence-survival (PRS) in comparison to no upgrading.

Methods: Methods: From initially 504 STS patients, 144 (median age at surgery: 65.6 [IQR: 52.9 – 74.9] years; 78 males [54.2%]) with primary localised STS of extremities and trunk, who had been treated with curative intent between 07/95 and 10/16 at a single tertiary sarcoma centre, and developed a local or distant recurrence during follow-up, were retrospectively included. Median follow-up from time of surgery until last follow-up or death was 34.5 (IQR: 12.5 – 89) months. Primary histology and grading (from naïve surgical specimen), as well as grading during first (and second) LR as well as DM were documented, together with potential changes in grading. Patients with and without changes in grading were compared with regards to PRS (based on the first recurrent event in case of both LR and DM) using log-rank test with last follow-up or death as endpoint.

Results: Thirty patients developed LR, 88 patients DM, and 26 both LR and DM. In altogether 83 patients, information on change in grading was available, whilst in the remaining 61 patients, recurrence had only been verified on imaging, with no histological confirmation. Of the 83 patients with histological diagnosis of recurrence available, grading of primary tumour was G1, G2, and G3/high-grade per definition in 4 (4.8%), 18 (21.7%), and 61 (73.5%) individuals, respectively. Most common histological subtypes were myxofibrosarcoma (n=25; 30.1%) and MFH/UPS (n=17; 20.5%). Median time from surgery to any recurrence was 16 (6 – 32) months. Upon recurrence, change in grading was observed in 9 patients (10.8%), with 7 presenting with higher-grade lesions, and 2 with lower-grade lesions than the primary tumour. Five of the 7 “upgradings” were observed in LR, and 2 in DM. In 6 of 7 “upgradings”, primary tumour had been diagnosed as G2 (and recurrence as G3), whereas in one case, primary tumour grading had been G1. The two “downgradings” were found in LR (one primarily diagnosed leiomyosarcoma G1/2, LR diagnosed G1; one primarily diagnosed myxofibrosarcoma G3, LR diagnosed G2). There was no significant difference in PRS between patients without change in grading (3-year survival probability 52.6%) and those with “upgrading” (3-year survival probability 62.5%) or “downgrading” (3-year survival probability not reached; log-rank p=0.593).

Conclusion: The frequency of change in grading upon recurrence of STS is relatively low. Although “upgradings” are the logical consequence of recurrence, two “downgradings” in patients with LR were likewise observed. Bearing the limitations of a small patient number as well as sampling bias due to cases without histological workup of recurrences in mind, a change in grading does not seem to have an impact on post-recurrence survival.

(P 459) EXPERIENCE IN THE USE OF INDIVIDUALIZED 3D-IMPLANTS IN THE RECONSTRUCTION OF DEFECTS IN PATIENTS WITH TUMORS OF THE CHEST WALL

Alexandr Gennadievich Salkov, MD[1]; Aslan Valiev, PhD[1]; Nikolay Petrochenko, Candidate of Sciences[1]; Alexandr Shin, Candidate of Sciences[1]; Alexandra Fedorova, Candidate of Sciences[1]; Olga Efimenko, MA[2], (1) National Medical Research Center of Oncology named after N.N. Blokhin "of the Ministry of Health of the Russian Federation, Moscow, Moskva, Russia, (2) LLC TIOS, Moscow, Moskva, Russia

Objective: Primary tumors of the chest wall account for, according to a number of authors, from 1 to 5% in the total structure of neoplasms, while about 60% of them are malignant neoplasms (MN), which are represented by bone sarcomas (55% of cases) and soft tissues (45% of cases) [1, 2].
Metastatic lesions of the ribs and sternum, in contrast to primary tumors, are observed 2-4 times more often [5]. Almost 30% of lesions of the sternum and ribs are secondary [3], most often these are metastases of breast cancer (BC), prostate, lung, kidney, thyroid and ovary. The lesion can be localized both in the sternum in isolation, and with the involvement of the chest wall.[4] The great rarity of this pathology, the complexity of the anatomical localization predispose to great technical difficulties that arise in the combined treatment of these patients and technical problems that arise during the surgical removal of these formations. There are few data in the literature on this topic. [5, 6, 7, 8] The paper presents the first experience of the National Medical Research Center of Oncology in the treatment of patients with tumor lesions of the chest wall.

Methods: At the National Medical Research Center of Oncology named after N.N. Blokhin from 2019 to 2023, 52 surgical interventions were performed in patients with primary and metastatic tumors of the chest wall. Resections of the rib or ribs, sternum were performed with simultaneous reconstruction with local tissues, displaced flaps, as well as using various synthetic and metal materials.
The rapid development of additive technologies has made it possible to define a new stage in reconstructive plastic surgery and onko orthopedy. Since 2020, at the NMRC of Oncology named after N.N. Blokhin, 19 operations were performed using individualized 3D implants in patients with tumors of the chest wall.
All patients underwent replacement of the defect with an individualized implant made according to anthropometric data.

Results: Since 2019, 52 patients with tumor lesions of the chest wall have been operated in the Department of General Oncology. In 19 of them, the defect was replaced with an individual 3D titanium implant during extensive resections. Our study presents a rather heterogeneous group of patients, the most common tumor being chondrosarcoma (36%). 17 cases of primary malignant tumors (89%) and 2 patients with metastatic lesion of the sternum, when resection of the chest wall was required (11%). The operation was radical in 15 patients (78%). Five patients died from progression (26%), among which, at the time of surgery, three had distant metastases and the operation was symptomatic. The longest follow-up period in our study was 35 months.

Conclusion: The use of new surgical technology can significantly improve the results of treatment in patients with tumor lesions of the chest wall, however, the results obtained suggest that more careful selection of patients and a special multidisciplinary approach are required when determining indications for surgical treatment.

№	Diagnosis	Age	Distant Metastasis	Recurrence/Progression	Period of observation	Result
1	Chondrosarcoma of sternum	47	no	9 m/3 m	35 m	Died of desease (DOD)
2	Peripheral lung cancer	62	no	no	31 m	Observation
3	Chondrosarcoma of the fifth rib	65	no	no/21 m	31 m	Secondary operation for distant metastasis
4	Osteosarcoma of the sternum	63	yes	2 m/further progression	4 m	DOD
5	Pleomorphic soft tissue sarcoma	29	no	No/3 m	3 m	DOD
6	Radioinduced myxofibrosarcoma of soft tissues	52	no	9 m/12 m	19 m	Chemotherapy
7	Breast cancer Metastasis	50	yes	3 m/3 m	6 m	DOD
8	Chondrosarcoma of the 5th rib	31	no	no	18 m	Observation
9	Chondrosarcoma of the 5th rib	71	no	нет	18 m	Observation
10	Metastasis of thyroid cancer	55	yes	no/3 m	17 m	Radiation therapy for lesions
11	Pleomorphic soft tissue sarcoma	46	yes	no/3 m	17 m	Chemotherapy
12	Angiosarcoma of the mediastinum	55	no	no/3 m	9 m	DOD
13	Soft tissue fibrosarcoma	75	no	no	15 m	Observation
14	Chondrosarcoma of sternum	73	no	no	14 m	Observation
15	Osteosarcoma of the sternum	58	no	no	12 m	Observation
16	Ewing's sarcoma II rib	30	no	no	3 m	Observation
17	Soft tissue liposarcoma	34	no	no	3 m	Chemotherapy
18	Chondrosarcoma of the sternum	60	no	no	0 m	Observation
19	Chondrosarcoma of the 5th rib	41	no	no	0 m	Observation

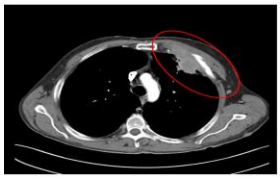


Fig. 1

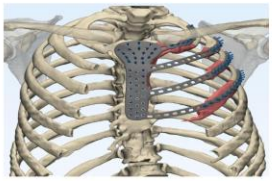


Fig. 2

An example of modeling an individual implant with determining the volume of resection (fig. 1 and 2)



Fig. 3

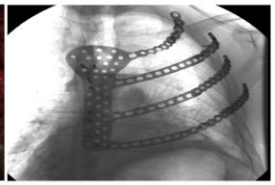


Fig. 4

The moment of implant installation (fig. 3), condition after surgery (fig. 4)

(P 460) IMPACT OF RESIDUAL TUMOR AFTER RE-EXCISION IN PATIENTS WITH PRIOR UNPLANNED RESECTION OF EXTREMITY AND TRUNK SOFT TISSUE SARCOMAS

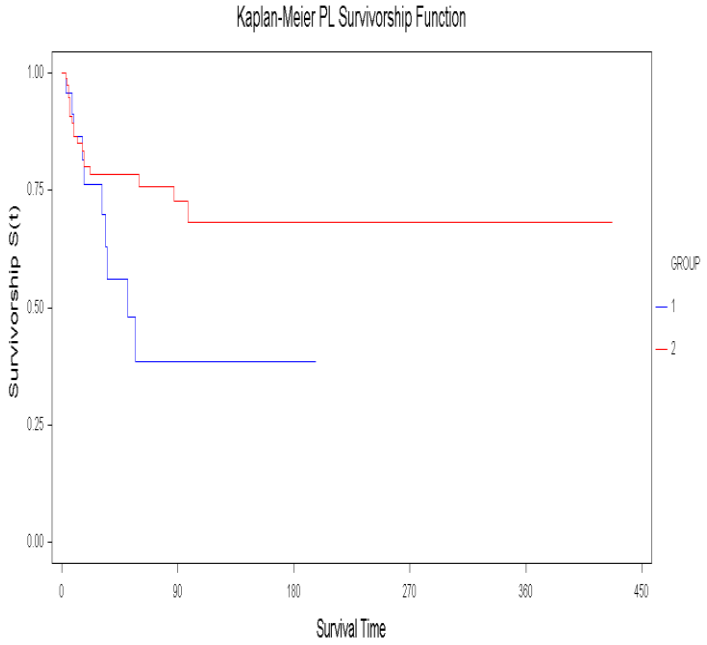
Sergio D. Quildrian, MD[1]; Walter S. Nardi, MD[2]; Anabela Daffinoti, MD[3]; Jorge A. Chapela, MD[3], (1) University of Buenos Aires, Angel H. Roffo Institute of Oncology - Buenos Aires British Hospital, Buenos Aires, Ciudad Autonoma de Buenos Aires, Argentina, (2) Buenos Aires British Hospital, Buenos Aires, Ciudad Autonoma de Buenos Aires, Argentina, (3) University of Buenos Aires, Angel H Roffo Institute of Oncology, Buenos Aires, Ciudad Autonoma de Buenos Aires, Argentina

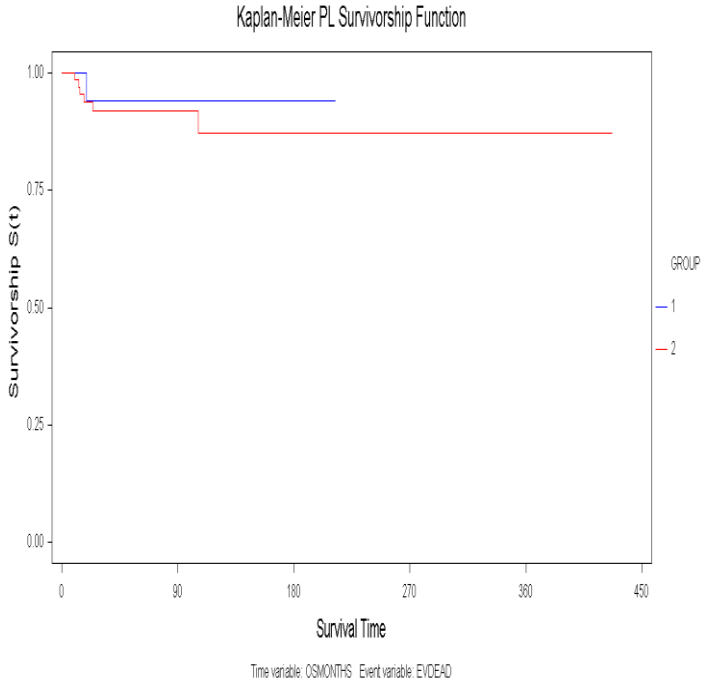
Objective: The majority of soft tissue sarcomas (STS) arise from extremities and trunk wall. In patients with localized disease, the recommended approach consists of complete en-bloc R0 surgical resection plus radiotherapy (in high-grade lesions, G2-3) after multidisciplinary tumor board evaluation. In patients with microscopically positive margin resection (R1), the probability of residual disease in tumor bed has led to evaluate the utility of re-resection. The aim of this study was to evaluate the impact of residual tumor in patients who underwent re-excision after unplanned R1 resection.

Methods: We analyzed retrospectively from a prospectively maintained database all patients (≥18yo) with extremity and trunk STSs treated in two referral centers in Argentina who underwent re-excision after prior R1 resection from January/2000 to December/2020. Demographic data and tumor characteristics were evaluated (location, size and neo/adjuvant treatment delivered). In order to compare oncologic results, the series was divided in two groups: G1 (residual tumor present in re-excision specimen) and G2 (no residual tumor). We evaluated overall survival (OS, death defined as event) and local recurrence-free survival (LRFS, recurrence defined as event). Survival was defined from time of re-excision to event detection.

Results: We identified 102 patients; median age 46(IQR, 31-59) years and 52(51%) patients were females. The tumor was located in trunk-wall in 30 (29.5%) patients and limbs in 70(70.5%) patients. Data on grade was available for 73 lesions and 63% (46/73) were high-grade. In 24 (23.5%) patients, residual tumor was detected after re-excision (Group 1) and the remaining 78 correspond to Group 2. After re-excision recurrence was observed in 10(41.6%) of 24 patients in Group 1, and 18 (23%) of 78 patients in Group 2 (p=0.12). Noteworthy 71% (20/28) of LRs happened in extremity locations and 65% (13/20) did not have RT. The 2-year and 5-year recurrence-free survival was 76% and 38% for Group 1 and 78% and 75% for Group 2 (p=0.08, Figure 1), respectively. The 5-year overall survival was 94% in Group 1 and 92% in Group 2 (p=0.58, Figure 2).

Conclusion: In our series, the presence of residual tumor after re-excision was observed in 23.5% of patients with prior unplanned R1 resection. We did not find a negative impact in terms of LRFS and OS when compared with patients without residual tumor. Nevertheless, the higher rate of recurrence in patients with residual tumor (G1) could lead to a potential more extensive and eventually radical resection, mainly in patients with extremity sarcomas, in case of late detection during follow up. The size of our series and the retrospective nature of this study avoid establishing a firm recommendation.


Kaplan-Meier PL Survivorship Function
Survivorship S(t)
1.00
0.75
0.50
0.25
0.00
0
90
180
270
360
450
Survival Time
GROUP
1
2
Time variable: RFSMONTHS Event variable: EVRECURRE


Kaplan-Meier PL Survivorship Function
Survivorship S(t)
1.00
0.75
0.50
0.25
0.00
0
90
180
270
360
450
Survival Time
GROUP
1
2
Time variable: OSMONTHS Event variable: EVDEAD

(P 461) OUTCOMES FOR OLIGOMETASTATIC PULMONARY DISEASE RECURRENCE TREATED WITH SURGERY ALONE FOR PATIENTS WITH SOFT TISSUE SARCOMAS A CASE SERIES

Priscila Barreto coelho, MD[1]; Brandon E. Rose, MD, MPH[2]; Steven Bialick, DO[3]; Raphael Yechieli, N/A, MD[4]; Dao Nguyen, MD[5]; Aditi Dhir, MD[6]; Gina Z. D'Amato, MD[5]; Jonathan Trent, MD, PhD[7]; Emily Jonczak, MD[8], (1) University of Miami, Miami, FL, (2) University of Miami and Jackson Health Systems, Miami Beach, FL, (3) Sylvester Comprehensive Cancer Center, University of Miami, Deerfield Beach, FL, (4) Sylvester Comprehensive Cancer Center, MIAMI, FL, (5) Sylvester Comprehensive Cancer Center, Miami, FL, (6) University of Miami Miller School of Medicine, Miami, FL, (7) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, (8) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL

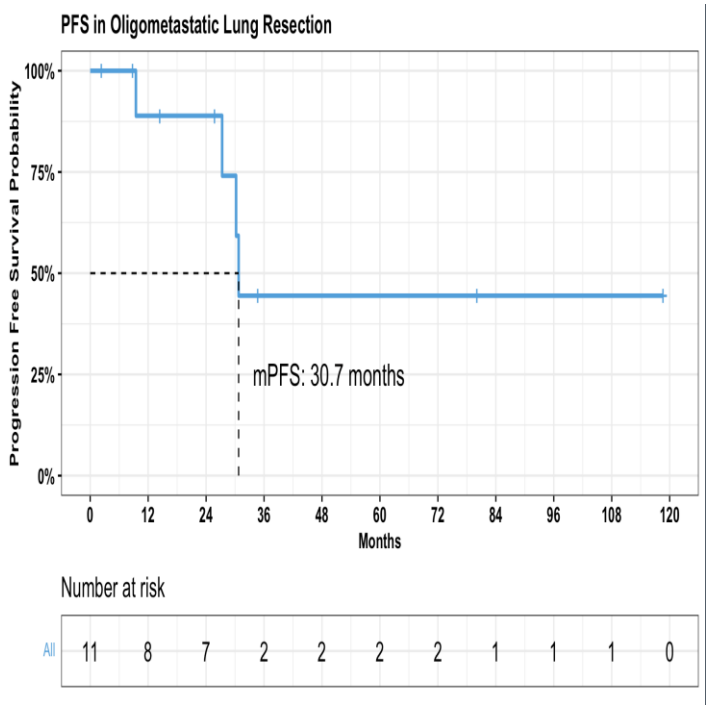
Objective: Oligometastatic disease refers to patients with metastases limited in number and organ sites, who may have a more indolent biology. Systemic therapy is generally used as a palliative measure for patients with STS initially presenting with metastatic disease or after distant relapse . The benefit of treating patients with systemic therapy for STS with oligometastatic recurrence amenable to resection or ablative therapies needs to be better understood. Identifying the patients most likely to benefit from local management alone is of the utmost importance in deciding whether or not to offer metastasis-directed therapy vs. systemic therapy vs. combined modality. We aimed to retrospectively look at patients in our institution with STS and oligometastatic pulmonary recurrence treated with surgical management only, and here we describe our findings.

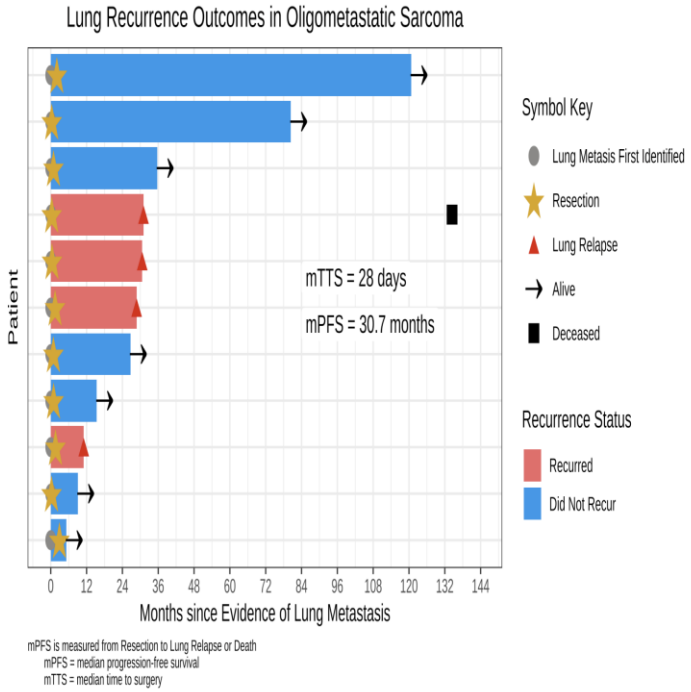
Methods: Under an IRB-approved protocol, we retrospectively reviewed patients diagnosed at our institution with STS who underwent the first treatment for their localized disease, followed by the development of metastatic disease to the lung, characterized as an oligometastatic disease recurrence managed with metastasectomy only. The European consensus definition used in this study was up to five metastases from up to three different sites. All data were retrieved from electronic medical records. The primary endpoint was investigator-assessed progression-free survival, and all analysis was done with “R” software.

Results: A total of 11 patients were identified to have been diagnosed with localized STS with subsequent oligometastatic pulmonary recurrence from January 2004 to December 2021, histology included: leiomyosarcoma (N=2), myofibroblastic sarcoma (N=1), pleomorphic myxofibrosarcoma (N=1), alveolar soft part sarcoma (N=1), synovial sarcoma (N=1), epithelioid hemangioepithelioma (N=1), rhabdomyosarcoma (N=1), extrskeletal osteosarcoma (N=1) and high grade sarcoma (N=2). All these patients underwent standard-of-care treatment for their localized disease that involved Surgery (Orthopedic, Reconstructive) with (N=9) or without (N=2) chemotherapy and radiation therapy. Median follow-up was 88 months (range 5.9 to 340). The median time to development of oligometastatic recurrence was 23.2 months. After the detection of the pulmonary metastasis, the mean time to surgical management (TSM) was 33.3 days, median TSM was 28 days (range 8- 88 days). Modalities of surgery were Video-Assisted Thoracic Surgery (VATS) (2/11, 18%) and thoracotomies (9/11, 82%). Four of the 11 patients developed another recurrence (36.4%) until data was collected on June 2023, and 7/11 (63.6%) had no recurrence. The median PFS was 30.7 months after the surgical management.

Conclusion: Oligometastatic pulmonary recurrence in patients with STS is an increasingly recognized tumor presentation with an opportunity for long-term tumor control with minimal side effects. A multidisciplinary approach is usually employed, combining surgery, radiation therapy,

and systemic treatment. Lung metastasectomy through either thoracotomy or VATS yields excellent results in patients with oligometastatic pulmonary disease recurrence. In our small series, median PFS was over 2.5 years which is more impressive than studies evaluating chemotherapy (single agent doxorubicin with mPFS of 2.52 months) and FDA-approved pazopanib (4.6 months). This data warrant the possibility for prospective studies evaluating the best management for this group of patients.





mPFS is measured from Resection to Lung Relapse or Death
mPFS = median progression-free survival
mTTS = median time to surgery

(P 462) EXTERNAL VALIDATION OF A NOMOGRAM PREDICTING THE RISK OF POSTOPERATIVE MAJOR WOUND COMPLICATION IN SOFT TISSUE SARCOMA FOLLOWING PREOPERATIVE RADIOTHERAPY AND RESECTION

Sarah E. Lindsay, MD[1]; Jacob Grassauer, BS[1]; Yee-Cheen Doung, MD[1]; Kenneth R. Gundle, MD[2]; James Hayden, MD, PhD[1]; Duncan Ramsey, MD, MS[1], (1) Oregon Health & Science University, Portland, OR, (2) Portland VA Medical Center, Portland, OR

Objective: Standard of care for soft tissue sarcoma (STS) is wide surgical resection combined with external beam radiation therapy (EBRT) and occasional chemotherapy. Unfortunately, postoperative surgical site complications and major wound complications (MWCs) are common in the setting of STS and are exacerbated by EBRT (particularly preoperative EBRT). While MWC have not been shown to affect long term recurrence or overall survival, these complications do contribute to overall morbidity.

Significant work has been done to identify risk factors associated with the development of MWC. Nomograms are useful disease-specific prognostic models that can be utilized to predict clinical outcomes. In 2022, Ouyang and colleagues developed a nomogram designed to predict the risk of MWC following preoperative EBRT and resection of STS of the trunk and extremity.1 In this cohort, 126 patients at a single institution met inclusion criteria and 19% experienced MWC. Age, tumor size, and metastasis at presentation were identified as independent risk factors for MWC. A nomogram was generated with a receiver operating characteristic curve (ROC) area under the curve (AUC) value of 0.86, suggesting that this model effectively predicts MWC. To date, this nomogram has not been externally validated.

The purpose of this study was to externally validate the nomogram generated by Ouyang and colleagues.

Methods: Institutional Review Board approval was obtained for this study. We retrospectively reviewed all patients at a single sarcoma center who underwent preoperative radiation followed by surgical resection for STS of the extremity or trunk between 2008 and 2022. Model variables were those proposed by the Ouyang nomogram: sex, age, body mass index, diagnosis of diabetes, presence of metastases on presentation, type of surgical closure, and tumor characteristics (site, size, volume, depth, grade, histological subtype). MWC were identified and were defined as any of the following within four months of initial resection: formal wound debridement in the operating room, procedural interventions such as percutaneous drainage, treatment with intravenous antibiotics, or deep wound packing for more than 120 days. Model discrimination (ROC and AUC), calibration, and overall performance (Brier score) were used to assess the model's performance.

Results: Two hundred seventy-nine patients were treated with preoperative radiation followed by surgical resection for management of STS of the extremity or trunk. Of these patients, 104 had MWC (37.3%).

The AUC was 0.633, suggesting overall poor discriminatory ability. The calibration curve demonstrated poor overlap between the model predictions and actual patient outcomes. Figures 1 and 2

A Brier score is a measure of how accurate predictions are compared to actual outcomes. A Brier score is on a scale of 0 to 1, and lower values are indicative of better model prediction performance. The Brier score for this model was 0.348, indicating poor model performance.

Conclusion: In external validation by a larger, independent cohort, the nomogram proposed by Ouyang performed significantly worse than on the training dataset. Additional work is needed to develop models predicting MWCs following STS resection.

Works Cited:

Ouyang, Z. et al. Nomogram Predicting the Risk of Postoperative Major Wound Complication in Soft Tissue Sarcoma of the Trunk and Extremities after Preoperative Radiotherapy. Cancers 14, 4096 (2022).

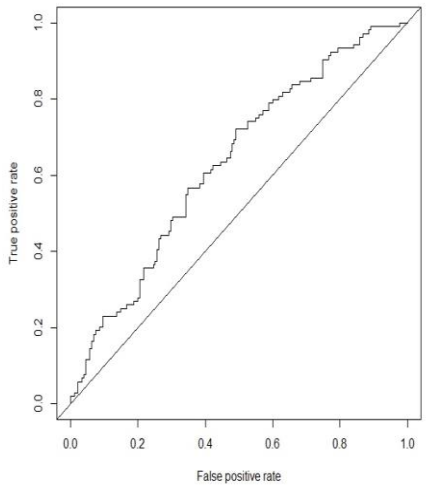


Figure 1: Receiver operating characteristic (ROC) curve area under the curve (AUC) of 0.633.

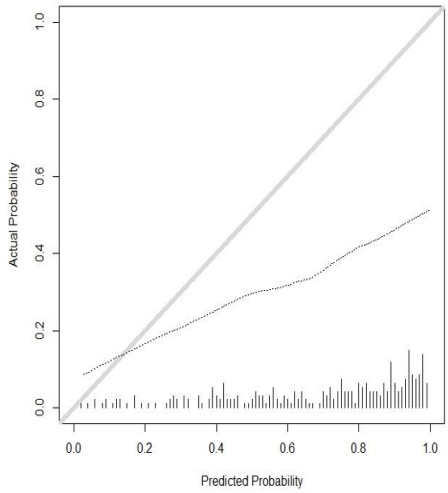


Figure 2: Calibration curve

(P 463) MACHINE LEARNING CORROBORATES TRADITIONAL REGRESSION TECHNIQUES IN PREDICTING MAJOR WOUND COMPLICATIONS AFTER SOFT TISSUE SARCOMA RESECTION WITHOUT IMPROVEMENTS IN PREDICTIVE PERFORMANCE

Sarah E. Lindsay, MD[1]; Jacob Grassauer, BS[1]; Spencer Smith, BS[1]; Steven Shelofsky, BS[2]; Jung Yoo, MD[1]; Yee-Cheen Doung, MD[1]; Kenneth R. Gundle, MD[3]; James Hayden, MD, PhD[1]; Duncan Ramsey, MD, MS[1], (1) Oregon Health & Science University, Portland, OR, (2) Oregon Health & Science University, West Linn, OR, (3) Portland VA Medical Center, Portland, OR

Objective: Postoperative major wound complications (MWCs) are common complications that occur after wide surgical resection of soft tissue sarcoma (STS). While infections have not been shown to affect oncologic outcomes, they are associated with substantial morbidity. Significant work has been directed at identifying risk factors associated with the development of MWC. Risk factors identified in previous work include neoadjuvant external beam radiation therapy (EBRT), preoperative chemotherapy, diabetes, patient age, resection size, tumor size, lower extremity tumor location, and wound closure methods.

There is considerable complexity in the development of a MWC following STS resection, and the etiology may be multifaceted. Quantification of the relative importance of identified risk factors has not been achieved. Developing predictive models to better understand these risk factors is essential in setting the foundation of future work aimed at reducing the incidence of MWC

The purpose of this study was to utilize logistic regression and machine learning modeling to quantify the risk factors associated with the development of MWC following resection of STS.

Methods: Institutional Review Board approval was obtained for this study. We retrospectively reviewed all patients at a single sarcoma center who were treated for STS of the extremity or trunk between 2008 and 2022. Data collected included the following: sex, age, patient body mass index (BMI), tobacco use, diagnosis of diabetes, presence of metastases on presentation, type of surgical closure, and tumor characteristics (site, size, volume, depth, grade, histological subtype). Laboratory values at time of resection were collected. MWCs were identified and were defined as any of the following within four months of initial resection: formal wound debridement in the operating room, procedural interventions such as percutaneous drainage, treatment with intravenous antibiotics, or deep wound packing for more than 120 days.

Univariate, logistic regression, and machine learning (XGBoost) analyses were performed to identify risk factors for MWCs. Model performance was assessed by receiver operating characteristic area under the curve (AUC).

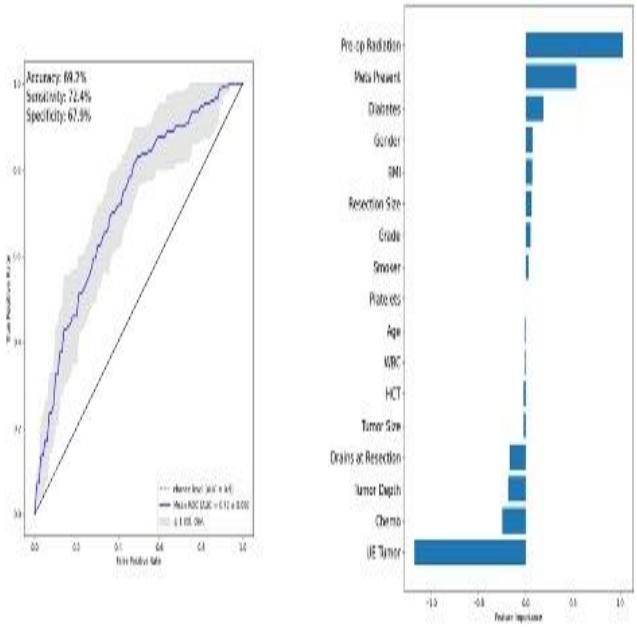
Results: Six hundred and seventy-seven patients were treated with wide surgical resection of STS between 2008 and 2022 at our institution. Of these patients, 177 had MWCs (26%). In the univariate analysis, the following factors were significantly associated with MWCs: BMI ($p< 0.0001$), diabetes ($p=0.03$), neoadjuvant radiation ($p< 0.0001$), lower extremity tumor location ($p< 0.0001$), presence of metastases on presentation ($p=0.005$), chemotherapy ($p=0.0017$), resection size ($p< 0.0001$), method of closure ($p=0.017$), drains ($p=0.0015$), and low platelet count (p-0.025).

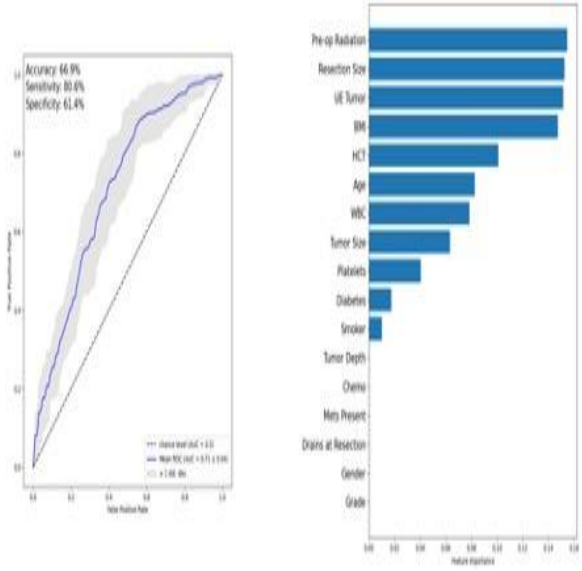
The sensitivity and specificity of the logistic regression model were determined to be 72.4% and 67.9% with an AUC of 0.72. The strongest risk factors for development of a MWC were

preoperative radiation and presence of metastases. Figure 1

The sensitivity and specificity of the XGBoost model were 80.6% and 61.4% respectively with an AUC of 0.71. The most important factors identified to be predictive for development of a MWC were preoperative radiation and resection size. Figure 2

Conclusion: Logistic regression modeling and machine learning-based regression models perform similarly. Both models identify preoperative radiation as the most important risk factor for MWCs following STS resection. Accurate modeling can guide efforts to minimize MWCs, and future efforts should be directed at improving and validating models.





(P 464) INDOCYANINE GREEN FLUORESCENCE IMAGING MAY DETECT TUMOUR RESIDUALS DURING SURGERY FOR BONE AND SOFT-TISSUE TUMOURS

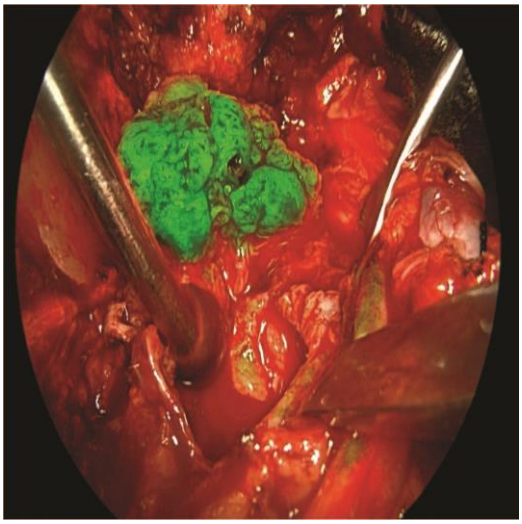
Xiaodong Tang, M.D.; Han Wang, M.D.; Tao Ji, MD; Huayi Qu, M.D.; Taiqiang Yan, M.D.; Dasen Li, M.D.; Wei Guo, N/A, M.D., Peking University People's Hospital, Beijing, Beijing, China (People's Republic)

Objective: The aim of this study was to determine the rate of indocyanine green (ICG) staining of bone and soft-tissue tumours as well as the stability and accuracy of ICG fluorescence imaging in detecting tumour residuals during surgery for bone and soft-tissue tumours.

Methods: ICG fluorescence imaging was performed during surgery in 34 patients with bone and soft-tissue tumours. ICG was administered intravenously at a dose of 2 mg/kg over a period of 60 minutes on the day prior to surgery. The tumour stain rate and signal-to-background ratio of each tumour were post hoc analyzed. After tumour resection, the tumour bed was scanned to locate sites with fluorescence residuals, which were subsequently inspected and biopsied.

Results: The overall tumour stain rate was 88% (30/34 patients), and specific stain rates included 90% for osteosarcomas and 92% for giant cell tumours. For malignant tumours, the overall stain rate was 94%, while it was 82% for benign tumours. The ICG tumour stain was not influenced by different pathologies, such as malignant versus benign pathology, the reception (or lack thereof) of neoadjuvant chemotherapies, the length of time between drug administration and surgery, the number of doses of denosumab for patients with giant cell tumours, or the tumour response to neoadjuvant chemotherapy. The overall accuracy rate of successfully predicting tumour residuals using fluorescence was 49% (23/47 pieces of tissue). The accuracy rate after en bloc resection was significantly lower than that after piecemeal resection (16% vs 71%; $p < 0.001$).

Conclusion: A high percentage of bone and soft-tissue tumours can be stained by ICG and the tumour staining with ICG was stable. This approach can be used in both benign and malignant tumours, regardless of whether neoadjuvant chemotherapy is adopted. The technique is also useful to detect tumour residuals in the wound, especially in patients undergoing piecemeal resection.





(P 465) UNPLANNED EXCISION OF SYNOVIAL SARCOMA: FACTORS ASSOCIATED WITH RECURRENCE AND SURVIVAL

Samuel Broida, N/A, MD[1]; Mikaela Sullivan, MD[2]; Alexandra Arguello, MD[2]; Steven Robinson, MBBS[2]; Scott Okuno, M.D.[2]; Brittany Siontis, MD[2]; Ivy A. Petersen, MD[2]; Safia K. Ahmed, MD[3]; Meng Xu-Welliver, MD, PhD[2]; Matthew T. Houdek, MD[2], (1) Mayo Clinic (Rochester), Rochester, MN, (2) Mayo Clinic, Rochester, MN, (3) Mayo Clinic Arizona, Phoenix, AZ

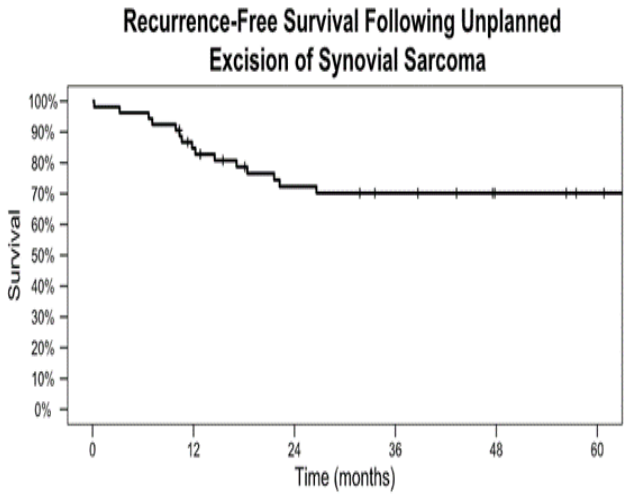
Objective: Synovial sarcoma is rare and may present as a small, slow-growing mass. These tumors are often mistaken as benign and are therefore prone to unplanned and/or non-oncologic excision. We sought to identify the rate of unplanned excision of synovial sarcoma and risk factors for recurrence and survival among this cohort.

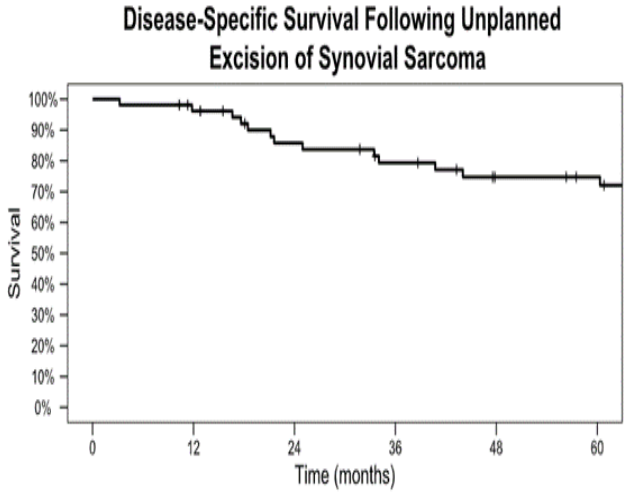
Methods: The medical records of 160 patients evaluated at a single institution for synovial sarcoma between 1997 and 2021 were retrospectively reviewed. Of these, fifty-three (33%) who underwent unplanned, non-oncologic excision of synovial sarcoma were included. Mean age of the cohort was 37±16 years. Primary lesions were located in the extremity (n=36), abdomen (n=4), thorax (n=7), or head/neck (n=6). The medium maximum pre-treatment dimension of the primary lesion was 3.6cm (range 0.9-14). Fifty (94%) patients underwent re-excision of the tumor bed, 25 received chemotherapy and 39 received radiation therapy. Univariate and multivariate analysis were performed to analyze the effect of risk factors on recurrence-free survival (RFS) and disease-specific survival (DSS).

Results: Twenty-three patients (46%) had no residual tumor on re-excision, all but two of which had received pre-operative radiotherapy. Four patients (two abdominal, one mediastinal, and one popliteal fossa tumors) had positive margins after re-resection. Three of these (two abdominal, one mediastinal tumors) patients developed local recurrence followed by metastatic disease and subsequent death. Four additional patients (9%) developed local recurrence

despite margin-negative resection, and three of these patients developed metastases followed by death. Of patients who did not develop local recurrence following margin-negative re-resection, 14% (n=6 of 44) developed metastases; all of these patients subsequently died of their metastatic disease. All three patients who did not undergo re-excision developed recurrence or succumbed to their disease. In the overall cohort, RFS at 1 and 5 years was 85% and 70%, respectively (Figure 1). DFS at 1 and 5 years was 96% and 75%, respectively (Figure 2).
On univariate analysis, positive prognostic indicators for recurrence and survival included extremity location, absence of bone involvement, size < 5cm, perioperative radiation, and absence of residual tumor on re-excision ($p < 0.05$). When controlling for tumor size and presence of residual tumor, the use pre-operative radiotherapy was associated with lower RFS (HR 0.18, 95% CI [0.04, 0.77], p=0.02) and DSS (HR 0.17, 95% CI [0.04, 0.81], p=0.03) on multivariate analysis. Chemotherapy was not associated with improved outcomes on univariate or multivariate analysis ($p>0.1$)

Conclusion: One-third of patients presenting with synovial sarcoma were diagnosed after unplanned, non-oncologic excision. Patients with large, truncal tumors had worse survival. Approximately half of patients who underwent unplanned excision had no residual tumor after pre-operative radiation. The use of radiation was associated with improved rates of recurrence and disease-specific death. Our results suggest that margin-negative re-resection and pre-operative radiotherapy should be considered when feasible following unplanned excision of synovial sarcoma.





(P 466) INTRAOPERATIVE EVALUATION OF BONE AND SOFT TISSUE SARCOMA SURGICAL MARGINS WITH INDOCYANINE GREEN FLUORESCENCE IMAGING

Matthew F. Gong, MD[1]; William T. Li, MD[2]; Sumail Bhogal, MD[2]; Brittany J. Royes, N/A, MS[1]; Tanya Heim, M.S.[1]; Marcus Malek, MD[3]; Rajeev Dhupar, MD[2]; Stella J. Lee, MD[2]; Richard L. McGough, III, MD[2]; Kurt R. Weiss, MD[1], (1) University of Pittsburgh, Pittsburgh, PA, (2) University of Pittsburgh Medical Center, Pittsburgh, PA, (3) Children's Hospital of Pittsburgh, Pittsburgh, PA

Objective: Achieving negative surgical margins is the cornerstone of bone and soft tissue sarcoma management, with positive margins portending a high risk of local recurrence (LR) and overall poor prognosis. Indocyanine green (ICG) is a fluorophore dye which has been utilized intraoperatively to evaluate surgical margins in several malignancies, including breast and gastrointestinal cancers. Given the critical importance of achieving negative margins in sarcomas, ICG fluorescence imaging has the potential to become a valuable intraoperative margin assessment tool for these uncommon and difficult-to-treat cancers. This study sought to evaluate ICG fluorescence imaging's utility in bone and soft tissue sarcoma resection.

Methods: This prospective non-randomized clinical trial included patients with biopsy-confirmed bone or soft tissue sarcomas undergoing surgical resection. Exclusion criteria included age < 18 years, prior adverse reaction to iodine or fluorescein, and renal disease. Patients received an intravenous ICG infusion dosed at 2.0 mg/kg three hours preoperatively. Intraoperatively, the removed tumor and tumor bed were imaged using a SPY-PHI fluorescent camera (Stryker Co.; Kalamazoo, MI). ICG-positive margins were defined as regions of tumor bed with a fluorescence intensity ≥77% of the resected tumor's highest measured intensity (Image 1). Sensitivity and specificity were compared between ICG margins, final pathology, and

the surgeon's blinded clinical impression. Cases where ICG matched or did not match final pathology were then grouped and compared using Student's t-test and Fisher's exact test (alpha = 0.05).

Results: 37 sarcoma (7 bone, 30 soft tissue) patients have been enrolled (Figure 1). ICG margins matched final pathology in 67.6% (25/37) of cases, with 23.1% sensitivity and 91.7% specificity (Figure 2). Surgeon impression matched final pathology in 59.4% (22/37) of cases, with 15.4% sensitivity and 83.3% specificity. No significant differences were found in ICG infusion time, infusion to incision time, or dosage between cases when ICG correctly or incorrectly matched final pathology (Figure 3). The presence of pre-operative radiation (10/37), pre-operative chemotherapy (5/37), or recurrent sarcoma (6/37) also did not significantly affect ICG margins when compared to final pathology margins.

Conclusion: ICG fluorescence imaging shows potential as an intraoperative margin assessment tool in sarcoma resection. Larger sample sizes and longer follow-up are needed to optimize dosing, infusion timing, and disease factors influencing this technique's accuracy in sarcoma patients. Investigating the effects of histological subtype, chemotherapy, and radiation will also contribute to our understanding of how ICG fluorescence imaging can be optimized to help surgeons resect sarcomas effectively.

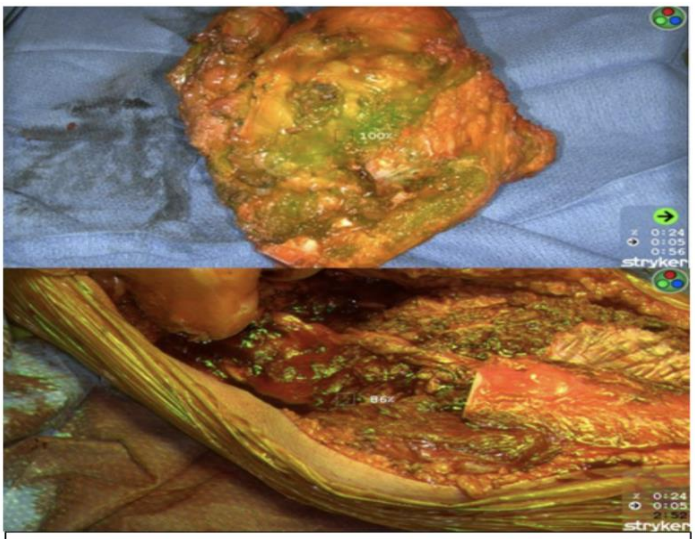


Image 1: Top - Calibration of fluorescence is performed in a patient with proximal tibia chondrosarcoma. The entire resected tumor is scanned and the brightest region of fluorescence is calibrated to 100%. Bottom - The entire tumor bed is scanned for fluorescence, with areas measuring 77% or more denoting a positive margin. 86% fluorescence was scanned in this case, denoting a positive ICG margin.

Figure 1. Demographics of patients (n = 37)	
Age (years)	57.4 ± 17.6
Gender	15 female (40.5%), 22 male (59.5%%)
Sarcoma Type	30 soft tissue (81.1%), 7 bone (18.9%)
Local Recurrence Case	6/37 (16.2%)
Prior Chemotherapy	5/37 (13.5%)
Prior Radiation	10/37 (27.0%)

Figure 2. Sensitivity, specificity, and predictive values compared to final pathology. PPV, positive predictive value; NPV, negative predictive value.

Margin Evaluation	Sensitivity (%).	Specificity (%).	PPV (%).	NPV (%).
ICG	23.1	91.7	60.0	68.8
Surgeon Impression	15.4	83.3	33.3	64.5

Figure 3. Comparison between matched and unmatched ICG cases with final pathology.

Variable	Matched (n = 25)	Unmatched (n = 12)	Significance
Infusion Time (min)	45.4 ± 14.7	40.1 ± 5.0	p = 0.25
Infusion to Incision Time (min)	139.1 ± 79.8	129.0 ± 61.0	p = 0.71
ICG dose (mg)	201.8 ± 49.7	195.8 ± 49.8	p = 0.73
Local Recurrence Case	4/25 (16.0%)	2/12 (16.7%)	p > 0.99
Prior Chemotherapy	3/25 (12.0%)	2/12 (16.7%)	p > 0.99
Prior Radiation	6/25 (24.0%)	4/12 (33.3%)	p = 0.70

(P 467) DOES PERIOPERATIVE RADIATION AFFECT IMPLANT SURVIVORSHIP OF PRIMARY TOTAL HIP ARTHROPLASTY IN THE SETTING OF METASTATIC BONE DISEASE: A MULTICENTER STUDY

Gayathri Vijayakumar, BS[1]; Alex Tang, MD[2]; Dylan Vance, BS[3]; Austin Yu, BS[4]; Richard S. Yoon, MD[2]; Kyle Sweeney, MD[3]; Alan T. Blank, MD, MS[5], (1) Rush University Medical Center, Edison, NJ, (2) Cooperman Barnabas Medical Center/Jersey City Medical Center – RWJBarnabas Health, Jersey City, NJ, (3) University of Kansas Medical Center, Kansas City, KS, (4) Rush University Medical Center, Alhambra, CA, (5) Rush University Medical Center, Chicago, IL

Objective: Metastatic bone disease (MBD) commonly affects the hip and surgical intervention including total hip arthroplasty (THA) is often indicated to treat the joint and improve function. Patients with metastatic cancer often receive radiotherapy and orthopedic oncologists must consider surgical risks with operating on irradiated bone and soft tissue. We evaluated surgical outcomes and implant survival of titanium acetabular components in patients treated for MBD in the setting of perioperative radiation.

Methods: This was a retrospective review of patients who underwent THA for MBD at three institutions between 2017 and 2021. Outcomes included rates of reoperation, complications, implant survival (IS), and overall survival.

Results: Forty-six patients who received primary THA for MBD were included in the study. Twenty patients (43.5%) received perioperative radiation for MBD. Six postoperative complications including one superficial wound infection, two dislocations, two pathologic fractures and one aseptic acetabular component loosening led to five reoperations. There were

no significant differences in postoperative outcomes, reoperation after THA, and IS based on radiotherapy status.

Conclusion: To our knowledge, this is the first paper evaluating primary THA outcomes and IS between patients who receive perioperative radiation for MBD to the hip and those who do not. As surgical management is a crucial part of the treatment in alleviating pain and disability in patients with MBD, we continue to recommend THA for patients who received radiation to the operative site.

Table 1. Postoperative Outcomes based on Radiotherapy Status

	p-value	Perioperative Radiation	No Radiation
Total Patients		20	26
Postoperative Complications	p=0.380	4 (20%)	2 (7.7%)
Superficial Wound Infection		0	1
Dislocation		2	0
Pathologic fracture		1	1
Aseptic acetabular component loosening		1	0
Reoperation after THA	p= 0.178	4 (20%)	1 (3.8%)
Overall Survival, months mean (95% CI)	p=0.921	25.90 (15.49-36.32)	20.79 (14.39-27.20)
Implant Survival, months mean (95% CI)	p=0.079	38.58 (28.70-48.45)	35.70 (31.42-39.98)

THA, total hip arthroplasty; CI, confidence interval

Table 2. Overall Survival and Implant Survival

	Univariate Analysis	Multivariate Analysis
Overall Survival		
Mean, months (95% CI)	24.75 (18.12-31.38)	
Chemotherapy	p= 0.428; HR 1.48 (95% CI 0.55-3.99)	
Perioperative Radiation	p= 0.921; HR 1.04 (95% CI 0.46-2.38)	
NLR	**p=0.026**; HR 1.07 (95% CI 1.01-1.13)	p= 0.250; HR 1.04 (95%CI 0.98-1.10)
Albumin	**p=0.002;** HR 0.34 (95% CI 0.18-0.67)	**p= 0.007**; HR 0.38 (95%CI 0.19-0.76)
Implant Survival		
Mean, months (95% CI)	43.47 (38.06-48.89)	
Chronic Steroids	**p=0.031**; HR 12.06 (95% CI 1.25-116.50)	
Perioperative Radiation	p=0.079; HR 7.14 (95% CI 0.80-64.08)	
Acetabular Component (porous titanium vs plasma spray)	p=0.786; HR 0.74 (95% CI 0.08-7.11)	
Femoral Component (cemented vs cementless)	p=0.163; HR 4.20 (95% CI 0.47-37.68)	

CI, confidence interval; NLR, neutrophil lymphocyte ratio; statistically significant p values are bolded

(P 468) OPIATE USE AFTER TOTAL HIP ARTHROPLASTY FOR METASTATIC BONE DISEASE

Gayathri Vijayakumar, BS[1]; Dylan Vance, BS[2]; Austin Yu, BS[3]; Matthew Colman, MD[4]; Steven Gitelis, MD[4]; Kyle Sweeney, MD[2]; Alan T. Blank, MD, MS[4], (1) Rush University Medical Center, Edison, NJ, (2) University of Kansas Medical Center, Kansas City, KS, (3) Rush University Medical Center, Alhambra, CA, (4) Rush University Medical Center, Chicago, IL

Background: Metastatic bone disease (MBD) commonly affects the hip, and surgical intervention including total hip arthroplasty (THA) may be indicated for pain relief or functional improvement. Following THA, patients are often prescribed short courses of opioids for postoperative pain relief. This study investigated postoperative opioid use and ambulation functional status following THA in MBD patients.

Methods: This was a retrospective review of patients using opioids postoperatively who underwent THA for MBD at two institutions between 2009 and 2022. Preoperative and postoperative opioid usage and ambulation status at 6 weeks, 90 days, 6 months, and 1 year were collected. Opioid usage and ambulation status changes postoperatively were compared using the sign test. Factors associated with postoperative opioid use at 6 weeks and 90 days were compared using $\chi 2$ test or Fisher's exact test.

Results: Twenty-seven primary THA and thirteen THA with complex acetabular reconstruction were included. Thirty patients used opiates preoperatively. Opioid use at 90 days was statistically significant for association with opioid use at 6 weeks. There was a statistically significant decrease between preoperative and 6 month median daily morphine milligram equivalent for preoperative opioid users. There was no statistically significant change in

ambulation status.

Conclusion: o our knowledge, this is the first paper evaluating postoperative opioid use following primary THA in MBD patients. After THA in the setting of MBD, patients exhibited a significant decrease in postoperative opioid use at 6 months. Future studies with larger cohorts should characterize postoperative opioid use following joint arthroplasty in MBD patients.

Table 1. Postoperative Opioid Use

Opiate Use at 6 weeks	26 (86.7%)	p value
Daily MME at 6 weeks, median (IQR)	45 (18.75-101.25)	
ΔMME preop and 6 weeks, median (IQR)	0 (*-12.75-41.25)	p=0.186
Opiate Use at 90 days	23 (76.7%)	
Daily MME at 90 days, median (IQR)	30 (8.75-122.50)	
ΔMME preop and 90 days, median (IQR)	10 (*-12.75 – 63.75)	**p<0.001**

MME, morphine milligram equivalent; IQR, interquartile range; Δ, delta; *negative value in IQR denotes greater opioid use compared to preoperative opioid use; statistically significant p value is bolded

(P 469) IMPACT OF A SPECIALIZED OUTPATIENT CLINIC ON BONE MRTASTASIS AND ITS BURDEN ON SPINE SURGEONS

Hiroyuki Tsuchie, MD[1]; Michio Hongo, M.D.[2]; Yuji Kasukawa, M.D.[2]; Hiroyuki Nagasawa, MD[2]; Daisuke Kudo, M.D.[2]; Naohisa Miyakoshi, Prof.[2], (1) Akita University Graduate School Of Medicine, Akita, Akita, Japan, (2) Akita University Graduate School of Medicine, Akita, Akita, Japan

Objective: Advances in drug therapies have improved the prognosis of patients with advanced cancer in recent years, and the number of patients with bone metastasis is likely to increase with the prognosis of cancer. The most frequent site of metastases in the bone is the spine. Fracture-related pain and paralysis often necessitating emergency surgery by a spine surgeon because of an extension to the spinal cord greatly impair the quality of life. In recent years, researchers have reported on the need for multidisciplinary approach by diverse professionals, such as orthopedic surgeons, oncologists, and radiologists. However, it is difficult to hold frequent meetings because a multidisciplinary approach requires the participation and cooperation of numerous professionals. Therefore, instead of the multidisciplinary approach, we decided to open a specialized outpatient clinic on bone metastasis. We aimed to evaluate the usefulness of a specialized outpatient clinic on bone metastasis, initiated instead of the

multidisciplinary approach.

Methods: This retrospective study enrolled patients with vertebral metastasis of carcinoma, diagnosed and surgically treated by spine surgeons at our hospital between May 2017 and April 2021. All patients who received radiotherapy for vertebral metastasis before the surgery or had brain-derived neurological deficit that affected their gait function were excluded. We eventually included 31 patients with vertebral metastasis, undergoing surgical treatment (16 men and 15 women, mean age: 67.3 years, and range: 42–83 years). We inspected the patient records to collect information on their regarding age, sex, the location of the primary tumor, the site of spinal lesion, a history of primary tumor treatment, paralysis or bladder and rectal disorder, a history of denosumab treatment, the period from spine surgeon consultation to surgery, surgical method, surgery time, operation room leaving time, intraoperative bleeding volume, and preoperative embolization. The specialized outpatient clinic on bone metastasis was initiated at our hospital in May 2019. We divided the patients into two groups, namely 2 years until April 2019 (Pre group) and 2 years after May 2019 (Post group), and we compared the clinical characteristics and information on surgery between the groups. In addition, we excluded patients who had not been treated for original carcinoma at our hospital or those not diagnosed with the primary tumor, and we compared only patients who were being treated for primary tumors at our hospital. Furthermore, we examined factors that affected the period from consulting the spine surgeon to the surgery, in addition to those that delayed leaving the operating room.

Results: The mean period from spine surgeon consultation to the surgery for all patients was 9.3±10.6 days (range: 0–46 days). Eighteen patients were being treated for primary tumors at our hospital. The post group demonstrated a longer period from spine surgeon consultation to surgery than the pre group (p=0.0498). Only in patients treated for primary tumors at our hospital, the period from spine surgeon consultation to surgery in the post group was significantly longer than that in the pre group (p=0.0129). The multivariate logistic regression analysis revealed that the period from spine surgeon consultation to surgery was significantly associated with visits to the specialized outpatient clinic (p=0.0460). In contrast, no obvious factors affected the operation leaving time.

Conclusion: The specialized outpatient clinic on bone metastasis had the possibility of reducing the burden on spinal surgeons by providing a margin for patients with spinal metastasis who required surgery. Because the number of patients with bone metastases is expected to increase, it is important to create a new system that will lead to early intervention for patients with bone metastases.

(P 470) PROGNOSTIC SIGNIFICANCE OF HISTOLOGICAL SUBTYPE IN SOFT TISSUE SARCOMA WITH DISTANT METASTASIS

Hiroyuki Tsuchie, MD[1]; Makoto Emori, MD PhD[2]; Hiroyuki Nagasawa, MD[3]; Yasutaka Murahashi, M.D.[2]; Emi Mizushima, MD[4]; Junya Shimizu, M.D.[2]; Toshihiko Yamashita, President[2]; Naohisa Miyakoshi, Prof.[3], (1) Akita University Graduate School Of Medicine, Akita, Akita, Japan, (2) Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan, (3) Akita University Graduate School of Medicine, Akita, Akita, Japan, (4) Sapporo Medical University School of Medicine, Bologna, Emilia-Romagna, Italy

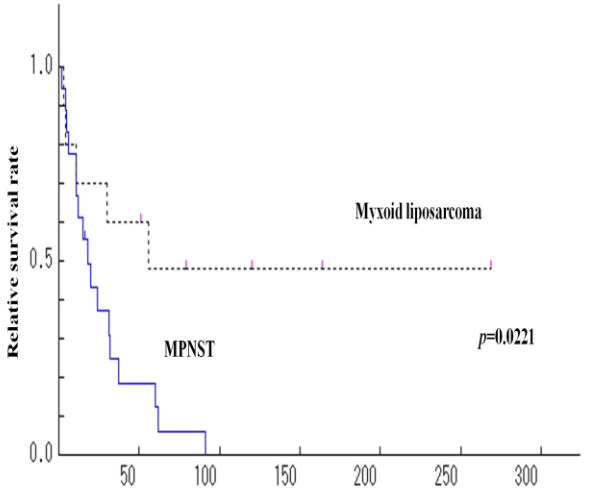
Objective: Soft tissue sarcoma (STS) is relatively rare and includes various histological subtypes., which results in relatively small populations of patients with each histological type. There is consensus that distant metastasis, which mainly involves the lungs, has a strong influence on prognosis. However, the small sample sizes for each subtype typically prevent studies from identifying a clear relationship between subtype and prognosis, despite the fact that several reports have examined the prognostic significance of the STS histological subtypes. Moreover, few studies have examined this topic among patients with STS and distant metastases. Therefore, we evaluated the relationship between the histological subtypes and clinical outcomes in STS patients with distant metastasis.

Methods: This retrospective study evaluated the histological subtypes of 105 patients with STS and distant metastasis treated at our two hospitals. Histological subtypes were considered relevant if we identified ≥ 5 cases per subtype, and included dedifferentiated liposarcoma, pleomorphic liposarcoma, myxoid liposarcoma, myxofibrosarcoma, undifferentiated pleomorphic sarcoma (UPS), synovial sarcoma, malignant peripheral nerve sheath tumor (MPNST), and leiomyosarcoma. The patients' records were searched to collect information regarding age, sex, histological subtype, anatomical location of the tumor, size, previous inappropriate excision, metastasis at diagnosis, stage of the primary tumor, treatments for the primary and metastatic tumors, follow-up period, and outcomes. Outcomes and clinical characteristics were compared according to the patients' histological subtypes.

Results: The histological diagnoses were dedifferentiated liposarcoma in 13 cases (12.4%), pleomorphic liposarcoma in 5 cases (4.8%), myxoid liposarcoma in 10 cases (9.5%), myxofibrosarcoma in 11 cases (10.5%), UPS in 31 cases (29.5%), synovial sarcoma in 11 cases (10.5%), MPNST in 18 cases (17.1%), and leiomyosarcoma in 6 cases (5.7%). Eighty-four patients (80%) developed pulmonary metastases. Patients with synovial sarcoma were significantly younger than those with dedifferentiated liposarcoma ($p< 0.01$), pleomorphic liposarcoma ($p< 0.01$), or UPS ($p< 0.01$). Dedifferentiated liposarcoma was significantly larger than pleomorphic liposarcoma ($p< 0.05$). Dedifferentiated liposarcoma was significantly more histologically malignant than myxoid liposarcoma ($p< 0.01$) or leiomyosarcoma ($p< 0.05$). The Kaplan-Meier curves for overall survival revealed that myxoid liposarcoma had a significantly better prognosis than MPNST ($p< 0.05$) (Figure 1). In the multivariate logistic regression analyses, the independent predictors of a poor prognosis were large size, advanced stage, and non-surgical treatment for metastasis ($p< 0.05$).

Conclusion: Myxoid liposarcoma had a better prognosis than MPNST in STS patients with distant metastasis. Although the surgical removal of distant metastatic lesions may improve the patient's prognosis, the location of the distant metastasis was not a significant factor.

Kaplan-Meier overall survival curves for the myxoid liposarcoma and malignant peripheral nerve sheath tumor (MPNST)



Number at risk

Myxoid liposarcoma	10	6	3	2	1	1	0
MPNST	18	3	0	0	0	0	0

(P 471) IMPACT OF PRIMARY TUMOR RESECTION ON METASTASIS TO THE LUNG IN PATIENTS WITH BONE AND SOFT-TISSUE SARCOMA

Hiroyuki Tsuchie, MD[1]; Makoto Emori, MD PhD[2]; Hiroyuki Nagasawa, MD[3]; Yasutaka Murahashi, M.D.[2]; Emi Mizushima, MD[4]; Junya Shimizu, M.D.[2]; Toshihiko Yamashita, President[2]; Naohisa Miyakoshi, Prof.[3], (1) Akita University Graduate School Of Medicine, Akita, Akita, Japan, (2) Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan, (3) Akita University Graduate School of Medicine, Akita, Akita, Japan, (4) Sapporo Medical University School of Medicine, Bologna, Emilia-Romagna, Italy

Objective: The presence of distant metastases, particularly in the lungs, strongly influences prognosis in patients with bone and soft-tissue sarcomas. Chemotherapy is commonly used in patients with bone and soft-tissue sarcomas with lung metastases, and the primary tumor is not aggressively resected in these patients. However, if the primary tumor interferes significantly with the patient's quality of life in terms of pain, neuropathy, and breaking of the skin, resection or even amputation may be required. Very few reports have investigated the effect of primary tumor resection on the prognosis of patients with bone and soft-tissue sarcomas with distant metastases. Furthermore, no reports have examined how excision of the primary tumor affects metastasis to the lungs. Therefore, we evaluated the impact of primary tumor resection on lung metastasis and prognosis in patients with bone and soft-tissue sarcoma.

Methods: We retrospectively identified 48 patients with bone and soft-tissue sarcoma involving the extremities or trunk with lung metastasis at the first visit treated at our two hospitals. Patients with lung metastasis confirmed at the same time as local primary tumor recurrence were also included. Since we aimed to assess the impact of primary tumor treatment, those who did not receive radiotherapy or surgical treatment for lung metastasis were also included.

Patients' records were searched to collect data regarding age, sex, malignancy and histological subtype, anatomical tumor location, size, lung and other metastasis, primary tumor treatment, follow-up period, and outcomes. The maximum diameter and the number of lung metastases at the first visit were evaluated. In addition, the maximum diameter of lung-metastatic lesions in each of the first and last chest computed tomography images were measured, and the average rate of increase per month (mm/month) was defined. Clinical characteristics and outcomes were compared based on primary tumor resection status, and the factors affecting prognosis were examined. In addition, we evaluated the factors affecting the average rate of increase in the size of lung metastases. We were able to measure the rate of increase in size of lung metastases more than once in 39 of the patients. The mean rate was 4.08 ± 5.37 mm/month and the patients were divided into fast and slow increase groups based on this average.

Results: Surgical treatment for the primary tumor was performed in 13 patients (27.1%), and radiotherapy for the primary tumor was performed in 16 patients (33.3%). In terms of outcomes, six patients were alive with the disease and 42 patients had died because of it at the last follow-up. Patients who had their primary tumor resected had significantly smaller lung metastases at the first visit and a slower rate of increase than those who did not undergo surgical resection ($p=0.0068$ and $p=0.0142$, respectively). The multivariate analysis revealed that the maximum diameter of lung metastases and the average rate of increase were significant prognostic predictors ($p=0.04$ and $p=0.0003$, respectively), yet primary tumor resection did not affect the outcome. The Kaplan–Meier curve revealed that patients with slow-growing lung metastases had a significantly better prognosis than those with fast-growing ones ($p=0.0003$). Patients in the fast-growing group had a significantly larger maximum diameter of lung metastasis at the first visit ($p=0.0419$). The multivariate logistic regression analysis revealed that the rate of increase was significantly associated with the maximum diameter of lung metastasis at the first visit ($p=0.0245$) and that surgical resection of the primary tumor did not affect the rate of increase.

Conclusion: Primary tumor resection of bone and soft-tissue sarcoma in patients with lung metastasis was not shown to affect their prognosis. The primary tumor should be excised only if it is necessary to improve the patient's quality of life.

(P 472) ICONIC (IMPROVING OUTCOMES THROUGH COLLABORATION IN OSTEOSARCOMA). AN EARLY LOOK AT SURGICAL DATA

Craig Gerrand, MD, FRCS Ed (Tr and Orth)[1]; Kenneth Rankin, FRCS[2]; Jonathan D. Stevenson, MBChB FRCS (Tr&Orth)[3]; Michael Parry, FRCS[4]; William Wilson, TBC[5]; Sandra J. Strauss, MD PhD FRCP[6], (1) Royal National Orthopaedic Hospital, London, England, United Kingdom, (2) Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, England, United Kingdom, (3) Royal Orthopaedic Hospital, Birmingham, England, United Kingdom, (4) Royal Orthopaedic Hospital, BIrmingham, England, United Kingdom, (5) UCL Cancer Trials Centre, London, England, United Kingdom, (6) UCL Cancer Institute, London, England, United Kingdom

Objective: Osteosarcoma (OS) is a rare cancer with heterogeneity in presentation, treatment and outcomes. Surgery is essential for cure. Understanding variations and outcomes of surgery is essential to improving outcomes.

Methods: ICONIC (NCT04132895) is an observational prospective cohort study recruiting all UK patients with a new OS diagnosis with matched clinical, biological and PROM outcomes. This was a review of the available surgical data for patients recruited to February 2023.

Results: 219 patients have been recruited from 24 UK sites, median age 19 (range 3-83), 57% male. Femur (50%, 108/215), tibia/fibula (24%, 51/215) and humerus (9% 19/215) are the most common anatomical sites, though less usual sites are also represented (craniofacial (7%, 14/215), pelvis/sacrum (5% 10/215) and rib/chest wall (3%, 6/215)). Tumours in the pelvis, ribs and maxilla/mandible were associated with older age (Table 1).

Of 178 patients with surgical data available, 169 (95%) patients underwent surgery. Tumours in the pelvis/sacrum had the largest median diameter (129.5mm, compared to 120mm (0-900) overall), the lowest rate of resection (3/10 (30%) not resected) and the highest rate of amputation (3/7, 42%, compared to 36/178 (21%) overall). Amputation was performed in 1/13 (8%) humeral, 17/88 (19%) femoral and 14/45 (31%) of below knee tumours. The closest soft tissue margins were seen in the forearm and pelvis (< 1mm margin in 2/4 (50%) and 3/6 (50%) respectively vs 23% overall). Overall 37/96 patients had a >=2mm margin and 37/96 had a good response to chemotherapy (>=90% necrosis) (Table 1).

Conclusion: Preliminary analysis demonstrates the acquisition of a rich surgical dataset. Results are broadly in keeping with other osteosarcoma studies but raise early questions about amputation rate and treatment variation across sites. Tumours in the pelvis and sacrum remain challenging. Biological, treatment and patient outcome data will further enhance this valuable resource.

	All N=178	Femur N=92	Tibia/fibula/metatarsal N=45	Humerus N=13	Radius/ulna N=4	Pelvis/sacrum N=10	Rib/chest wall N=4	Maxilla/mandible N=8	Scapula/clavicle N=1
Patient had resection of primary tumour, N(%)									
Yes	169 (95%)	88 (96%)	45 (100%)	12 (92%)	4 (100%)	7 (70%)	4 (100%)	7 (88%)	1 (100%)
No	9 (5%)	4 (4%)	0	1 (8%)	0	3 (30%)	0	1 (13%)	0 (0%)
Surgery type									
Amputation	36 (21%)	17 (19%)	14 (31%)	1 (8%)	0	3 (43%)	0	0	0 (0%)
Limb salvage	116 (69%)	70 (80%)	29 (64%)	9 (75%)	4 (100%)	3 (43%)	0	0	1 (100%)
Other	17 (10%)	1 (1%)	2 (4%)	2 (17%)	0	1 (14%)	4 (100%)	7 (100%)	0 (0%)
Maximum tumour dimension - Overall (mm)									
Median (range)	120mm (0-900)	125mm (0-900)	110mm (0-650)	125mm (14-265)	92mm (0-130)	129.5mm (55-230)	105mm (10-135)	55mm (33-170)	80mm (80-80)
<=80mm	42 (25%)	16 (18%)	13 (29%)	3 (25%)	2 (50%)	1 (14%)	1 (25%)	5 (71%)	1 (100%)
>80mm	123 (73%)	71 (81%)	32 (71%)	8 (67%)	2 (50%)	5 (71%)	2 (50%)	2 (29%)	0 (0%)
Unknown	4 (2%)	1 (1%)	0	1 (8%)	0	1 (14%)	1 (25%)	0	0 (0%)
Narrowest bone margin (mm)									
Median (range)	42mm (0-450)	50mm (0-195)	42mm (0-450)	25mm (0-75)	23mm (20-50)	12.5mm (1-50)	10mm (0-40)	12mm (1-15)	0mm (0-0)
<50mm	88 (52%)	40 (45%)	24 (53%)	9 (75%)	3 (75%)	5 (71%)	3 (75%)	3 (43%)	1 (100%)
>=50mm	68 (40%)	43 (49%)	21 (47%)	2 (17%)	1 (25%)	1 (14%)	0	0	0 (0%)
Unknown	13 (8%)	5 (6%)	0	1 (8%)	0	1 (14%)	1 (25%)	4 (57%)	0 (0%)
Narrowest soft tissue resection margin (mm)									
<1mm	36 (21%)	15 (17%)	9 (20%)	4 (33%)	2 (50%)	3 (43%)	1 (25%)	1 (14%)	1 (100%)
<2mm	17 (10%)	8 (9%)	5 (11%)	2 (17%)	0	1 (14%)	0	0	0 (0%)
>=2mm	84 (50%)	52 (59%)	19 (42%)	4 (33%)	2 (50%)	2 (29%)	2 (50%)	3 (43%)	0 (0%)
Not stated	26 (15%)	11 (13%)	10 (22%)	1 (8%)	0	0	1 (25%)	3 (43%)	0 (0%)
Tumour at surface of specimen	4 (2%)	1 (1%)	2 (4%)	1 (8%)	0	0	0	0	0 (0%)
Unknown	2 (1%)	1 (1%)	0	0	0	1 (14%)	0	0	0 (0%)
Post-chemotherapy necrosis	N=122	N=64	N=35	N=8	N=3	N=4	N=2	N=5	N=1
Necrosis seen	117 (96%)	62 (97%)	34 (97%)	8 (100%)	2 (67%)	3 (75%)	2 (100%)	5 (100%)	1 (100%)
Median (range)	80% (5-100)	80% (5-100)	77.5% (5-99)	90% (40-99)	55% (50-60)	30% (30-40)	71% (50-92)	90% (50-95)	80% (80-80)
<90%	75/117 (64%)	42/62 (68%)	21/34 (62%)	4/8 (50%)	2/2 (100%)	3/3 (100%)	1/2 (50%)	1/5 (20%)	1/1 (100%)
>=90%	42/117 (36%)	20/62 (32%)	13/34 (38%)	4/8 (50%)	0/2 (0%)	0/3 (0%)	1/2 (50%)	4/5 (80%)	0/1 (0%)
Unknown	5 (4%)	2 (3%)	1 (3%)	0	1 (33%)	1 (25%)	0	0	0 (0%)

(P 473) GROIN PAINS: APPROACHES AND OUTCOMES FOR INGUINOFEMORAL SOFT TISSUE SARCOMAS

Alexandra C. Istl, MD, MPH[1]; Matthew Van Boxtel, BSc, MD[2]; Maxwell Cardwell, BA, MD[2]; Callisia Clarke, MD[3]; Anai Kothari, MD[1]; John C. Neilson, MD[1]; David King, MD[1]; Adam Wooldridge, MD[1]; Meena Bedi, MD, MS[1]; John Charlson, MD[4]; Peter Rossi, MD[2]; Krisztian Kovacs, MD[2]; John LoGiudice, MD[2], (1) Medical College of Wisconsin, Milwaukee, WI, (2) MCW, Milwaukee, WI, (3) Medical College Of Wisconsin - Surgical Oncology, Milwaukee, WI, (4) Medical College of Wisconsin, Wauwatosa, WI

Objective: Soft tissue sarcomas (STS) involving the groin and inguinal canal present diagnostic and therapeutic challenges. These can be diagnosed after incidental or incomplete resection resulting in delays to definitive care. Furthermore, sarcomas arising in or invading structures in the inguinofemoral region are more likely to require complex vascular resections and abdominopelvic reconstructions, outcomes of which are significantly impacted by perioperative therapy. This cohort study from a high-volume center aims to characterize the modes of diagnosis, diagnostic and therapeutic considerations, and outcomes for inguinofemoral sarcomas.

Methods: A single institution retrospective cohort study was conducted at a high-volume sarcoma center. Patients treated for STS involving the groin or inguinal canal from 2004 to 2022 were included. Data on patient demographics, family history, presenting symptoms, diagnostic approaches, treatment received, perioperative complications, and oncologic outcomes were collected. Actual wound complication rates compared to those predicted by a lower extremity sarcoma nomogram were reviewed. Descriptive statistics are presented as frequencies and measures of central tendency. Univariate and multivariate regression analyses were conducted to identify variables associated with complications and recurrence.

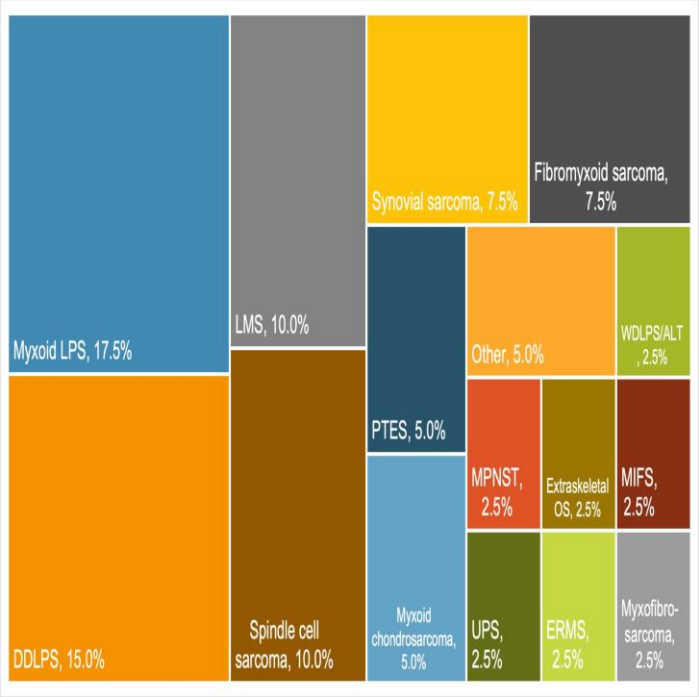
Results: Forty patients aged 25-96 years were included in our study. Median age at diagnosis was 56.6 years. Histologies are summarized in Figure 1. 100% of biopsies were reviewed by a sarcoma pathologist. The most common presenting symptoms were pain (46.2%) and palpable mass (59%); two were diagnosed incidentally after inguinal hernia surgery. 7.5% were recurrences from a previous or remote operation. Site of origin is summarized in Figure 2.

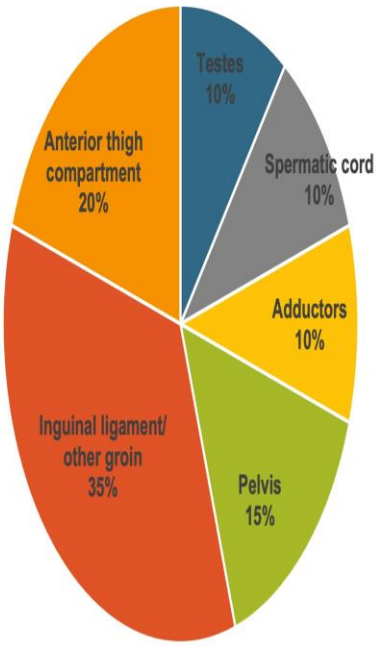
More than half of patients had a family history of cancer and 22.7% had >1 first-degree relative with a cancer diagnosis. Subjects had been treated at our institution prior to routine institutional germline genetic testing for sarcoma patients. However, three patients underwent genetic testing and two were found to have pathologic germline mutations (MSH3 and NF2).

75% of patients were treated with radiation (76.7% neoadjuvant) and 35% of patients were treated with chemotherapy (38.5% neoadjuvant). All resectable patients underwent surgery. Nine required vascular resection, 8 required orchiectomy, and 15 underwent an inguinal ligament, abdominal wall, or pelvic floor reconstruction. Thirty-day complication rate was 36.4% and 90-day complication rate was 39.4%. Anticipated risk of post-operative wound complications from validated nomogram data for lower extremity sarcoma (Bedi et al. 2020) ranged from 9-41% depending on patient data. The observed wound complication rate in our cohort was 24.2%. None of neoadjuvant radiation, chemotherapy, vascular resection, or abdominopelvic reconstruction were associated with postoperative complications on univariate or multivariate analysis.

Median duration of follow-up was 46 months (IQR 17-96), during which time 17.5% of patients developed a local recurrence (LR). Median time to LR was 5 months (IQR 2-18). Distant recurrence (DR) rate was 33% and median time to DR was 18 months (IQR 3-30). 20% of patients in the cohort died after presentation with metastatic disease or DR.

Conclusion: Inguinofemoral sarcomas present several challenges related to timely diagnosis and referral, perioperative therapy, complex surgical decision-making, and translation of these factors into oncologic outcomes. This study provides a granular foundation and direction for future studies, including an upcoming collaborative TARPSWG initiative, that will address unique facets of diagnosis, provider education, perioperative care, and optimizing of outcomes for this population.





Withdrawn ~~(P 474) PREDICTORS OF EARLY INTROWTH AND FAILURE FOLLOWING SHORT COMPRESS SPINDLE OSSEOINTEGRATION FIXATION AT THE LOWER EXTREMITIES~~

Withdrawn ~~(P 475) PRE-PLATING PRIOR TO INTERCALARY TUMOR RESECTION AND ALLOGRAFT RECONSTRUCTION: LESSONS LEARNED~~

Withdrawn ~~(P 476) FAVORABLE RESULTS FOLLOWING TREATMENT OF UNICAMERAL BONE CYSTS WITH DOXYCYCLINE SCLEROGRAFT TECHNIQUE~~

Withdrawn ~~(P 477) TREATMENT OF PROXIMAL FEMUR UNICAMERAL BONE CYSTS WITH DOXYCYCLINE SCLEROTHERAPY~~

Withdrawn ~~(P 478) TREATMENT OF CHONDROBLASTOMA WITH PERCUTANEOUS CRYOABLATION~~

Withdrawn ~~(P 479) EXPANDABLE ENDOPROSTHESES: SHOULD WE CONSIDER NARROWING OUR INDICATIONS? A SYSTEMATIC REVIEW OF THE LITERATURE AND COMPARISON OF OUTCOMES BY AGE GROUPS~~

Withdrawn ~~(P 480) TARGETED MUSCLE REINNERVATION FOR HIP DISARTICULATION - EVALUATION OF EARLY FUNCTIONAL OUTCOMES~~

Withdrawn ~~(P 481) **CUSTOM ADAPTERS FOR COMPRESS SPINDLE OSSEOINTEGRATION FIXATION IN REVISION LIMB SALVAGE SURGERY OF THE DISTAL FEMUR: A CASE SERIES**~~

Withdrawn ~~(P 482) **COMPARISON OF RECONSTRUCTIVE TECHNIQUES FOR METASTATIC DISEASE IN THE PROXIMAL HUMERUS**~~

Mikaela Sullivan, MD; Alexandra Arguello, MD; Peter S. Rose, MD; Matthew T. Houdek, MD, Mayo Clinic, Rochester, MN

Objective: The proximal humerus is a common location for metastatic disease, and numerous options are available for limb-salvage surgery in patients with impending or pathologic fracture. Endoprosthetic composites are often utilized for reconstruction with both hemiarthroplasty (HA) and reverse arthroplasty (RA) constructs being described. RA constructs have been shown to have improved functional outcomes in patients undergoing reconstruction for osseous lesions, but no studies have compared techniques in metastatic disease alone. The aim of this study is to compare functional outcomes and complications between HA and RA techniques in patients undergoing endoprosthetic reconstruction for proximal humerus metastases.

Methods: A total of 49 (25 male, 24 female) patients undergoing intraarticular resection of the proximal humerus for metastatic disease were retrospectively reviewed. Reconstructions included 31 HA constructs and 18 RA constructs. The majority (88%) of patients presented with pathologic fracture, with the most common diagnoses including renal cell carcinoma (47%), multiple myeloma (14%), and breast carcinoma (10%). All procedures were performed via the deltopectoral approach, and all implants were fixed via cementation. Average resection length was 11±3 cm, resection was above the deltoid insertion in 84% of cases, and the axillary nerve was preserved in 98% of cases. Average follow up was 2.6±3.5 years.

Results: Patients with RA reconstructions had significantly better post-operative forward elevation (73° vs. 37°, p=0.0005), though there was no difference in post-operative external rotation between groups. RA patients also had significantly higher Simple Shoulder Test scores (3.6 vs. 2.5, p=0.048). Patients with HA reconstruction experienced significantly more complications (n=18, 55% vs n=2, 9%, p=0.0014), with instability being the most common complication (n=14, 45% vs. n=2, 9%, p=0.0079). Two patients (both HA reconstructions) experienced a periprosthetic fracture about the distal implant. No patients underwent revision surgery.

Conclusion: In this series, patients with metastatic disease of the proximal humerus had improved post-operative range of motion as well as higher functional outcome scores when undergoing reconstruction with a reverse endoprosthetic construct compared to a hemiarthroplasty construct. For patients with metastatic disease of the proximal humerus requiring reconstruction, preference for reverse endoprosthetic composites should be given over hemiarthroplasty endoprosthetic composites.

(P 483) **DID THE COVID-19 PANDEMIC NEGATIVELY AFFECT OUTCOMES FOR PATIENTS WITH EXTREMITY SOFT TISSUE SARCOMAS?**

Anthony Griffin, MSc[1]; Kim Tsoi, MD, PhD[2]; Philip Wong, MD[3]; Charles Catton, MD[3]; Peter Chung, MBChB MRCP FRCR FRCPC[3]; David Shultz, N/A, MD[3]; Peter Ferguson, MSc., MD[2]; Jay Wunder, M.Sc, MD[2], (1) Mount Sinai Hospital, Toronto, ON, Canada, (2) University of Toronto, Toronto, ON, Canada, (3) Princess Margaret Cancer Centre, Toronto, ON, Canada

Objective: The COVID-19 pandemic has had a major impact on the ability of global health care systems to deliver timely and appropriate care. The goal of this study was to determine the impact of the COVID-19 pandemic on outcomes for patients with extremity soft tissue sarcoma treated at a tertiary care centre.

Methods: Patients treated surgically for an extremity STS from January 2017-May 2022 were identified from our prospectively collected database and divided into pre-COVID (Jan 2017-Feb 2020) and during COVID (March 2020-May 2022) groups. Patient demographics, tumour characteristics, surgical procedures, and outcomes were compared between the 2 groups. Categorical variables were compared with Chi-square test, means with t-test and local recurrence-free survival with Kaplan-Meier method.

Results: A total of 700 patients were identified: 426 pre-COVID and 274 during COVID. There was no difference in gender (female 181 (42.5%), 109 (40%, p=0.5); presentation with metastases (30 (7%), 19 (6.9%), p=0.7); prior 'whoops' surgery (74 (17.4%), 45 (16.4%), p=0.7); need for vascular (20 (4%), 11 (4%), p=0.7), nerve (14 (2.9%), 7 (2.6%), p=0.6) or bone (32 (6.7%), 20 (7.9%), p=0.9) resection; positive margins (81 (19%), 38 (14%), p=0.2); or tumour size (mean 9.3 cm, median 7.9 cm, range 0.5-42 cm; mean 9.8 cm, median 7.7, range 1-35.4 cm, p=0.19) between patients treated before or during the pandemic, respectively. There was a difference in the use of VAC for wound closure- 79 (18.5%) pre-COVID and 89 (32.5%) during COVID (p< 0.001). There was also a difference in wound complications: 24.5% pre-COVID compared to 17.9% during COVID (p=0.045). For patients treated with preoperative radiotherapy (224 pre-COVID, 139 during), the time from radiation completion to surgery was not different between the two time periods: pre-COVID a mean of 50 days, (median 49, range 18-149); while during COVID a mean of 50 days (median 45.5, range 13-169). Estimated 2-year local recurrence-free survival was 95.3% pre-COVID compared to 91.7% during COVID (log rank 5.6, p=0.018).

Conclusion: Surprisingly, patients with extremity STS did not present with larger tumours or a higher percentage with metastatic disease during the pandemic. VAC usage increased during the pandemic and a small decrease in local recurrence-free survival was seen. Follow-up for the COVID cohort is currently too short for a comparison of metastasis-free or overall survival between the 2 groups.

(P 484) OBESITY INCREASES THE RISK OF MAJOR WOUND COMPLICATIONS FOLLOWING PELVIC RESECTION FOR BONE SARCOMA

Patrick Qi Wang, MD, MSc, FRCSC[1]; Izuchukwu Ibe, MD[2]; Meshal Alfaraidy, MBBS, FRCSC[1]; Nicholas Eastley, MBChB, BMedSci, FRCS (Tr & Orth), PhD[1]; Jay Wunder, M.Sc, MD[1]; Peter Ferguson, MSc., MD[1]; Kim Tsoi, MD, PhD[1]; Anthony Griffin, MSc[3], (1) University of Toronto, Toronto, ON, Canada, (2) University of Mississippi, Jackson, MS, (3) Mount Sinai Hospital, Toronto, ON, Canada

Objective: Obesity in malignant bone tumours is a non-modifiable risk factor given the time sensitive nature of the disease. Elevated body mass index (BMI) increases the risk of surgical site infections and wound complications in limb bone sarcoma treatment. However, there is a paucity of data in the literature on the effect of obesity in patients who undergo pelvic resections. The objective of this study is to evaluate the association between obesity and major wound complications in pelvic bone sarcoma surgery.

Methods: Consecutive patients who underwent pelvic resection to treat bone sarcoma from 2005 to 2021 were reviewed from a prospectively collected single-centre database. The minimum follow-up was six months. Patients with benign tumours or primary soft tissue sarcomas were excluded. Patients were evaluated for major and minor wound complications. Major wound complication was defined as the need for a secondary surgical debridement procedure. Patients with minor wound complications only required bedside wound care with or without antibiotics. Primary outcomes were the effects of obesity on the rate of wound complications. Patients were classified according to their body mass index (BMI): obese (≥ 30kg/m2), overweight (25-29.9 kg/m2), healthy weight (18.5-24.9 kg/m2), underweight (< 18.5kg/m2). Secondary analysis was performed to identify any independent risk factors for wound complications.

Results: Among the 106 included patients, the number of obese, overweight, healthy weight, and underweight patients was 23, 35, 45, and three respectively. Twenty-seven patients sustained a major wound complication and eight patients were treated for a minor wound issue. The proportion of obese patients who sustained a major wound complication was significantly higher compared to overweight, healthy weight, and underweight patients (12/23 (52%), 6/35 (17%), 9/45 (20%), and 0/3 respectively, p = 0.0087). The odds ratio and relative risk of a major wound complication among obese patients were 4.95 and 2.89 respectively. Other factors including patient age, sex, tumour histology, tumour volume, tumour grade, type of reconstruction, type of closure (primary vs. soft tissue reconstruction), neo-adjuvant radiation therapy and/or chemotherapy did not significantly affect outcomes. The BMI status did not play a significant role in the rate of minor wound complications.

Conclusion: Obesity is associated with an increased rate of major wound complications requiring a secondary surgical debridement procedure in patients who undergo pelvic resection for bone sarcoma. This highlights the importance of careful intraoperative and perioperative wound management as well as patient education to optimize outcomes.

(P 485) SUBTROCHANTERIC NON-UNION AFTER INTRAMEDULLARY NAILING DUE TO PATHOLOGICAL FRACTURE:PROXIMAL FEMORAL REPLACEMENT AS A SALVAGE APPROACH

Moses Markowitz, BS[1]; David Constantinescu, MD[1]; Waleed Al-Hardan, MD[1]; Juan Pretell-Mazzini, MD[2]; Sheila Ann Conway, MD[3], (1) University of Miami Miller School of Medicine, Miami, FL, (2) Miami Cancer Institute, Miami, FL, (3) Wake Forest University School of Medicine, Winston Salem, NC

Objective: To assess proximal femoral replacement as a treatment solution for nonunion of pathologic subtrochanteric fractures after cephalomedullary nailing in patients with pathological fracture and previously irradiated bone.

Methods: Retrospective review of five patients with pathological subtrochanteric femoral fractures that were treated with cephalomedullary nailing and developed a nonunion, which was revised with conversion to a proximal endoprosthetic replacement between September 2020 and June 2021.

Results: Preoperative radiographs demonstrated hardware failure in all five patients (Figure 1). All five patients had previously been treated with radiation (Figure 2). One patient had latest follow-up at 2 months postoperatively. At that time, the patient was walking with a walker for assistance, with no evidence of hardware failure or loosening on imaging. The remaining four patients had latest follow-up ranging from nine to fifteen months after surgery. At their latest follow-up, three of the four patients were ambulatory with no pain, using only a cane for assistance with longer distances. The other patient demonstrated pain in his affected thigh, utilizing a walker for assistance with ambulation at latest follow-up, but not requiring further surgical interventions. There were no hardware failures or implant loosening on imaging at latest follow-up (Figure 3). None of the patients required revision and there were no postoperative complications observed at their last follow-up.

Conclusion: In patients with pathological fractures in the subtrochanteric region that are treated with cephalomedullary nailing and developed a nonunion; treatment with conversion to a proximal femoral replacement with a mega prosthesis is a valuable treatment with good functional results and low risk for complications.

Figure 1: Intraoperative radiographs from Patient 5 identifying fracture site and screw location

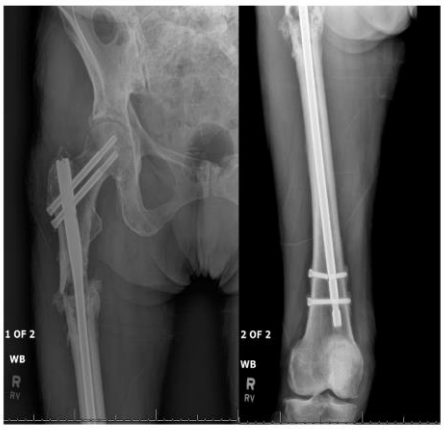
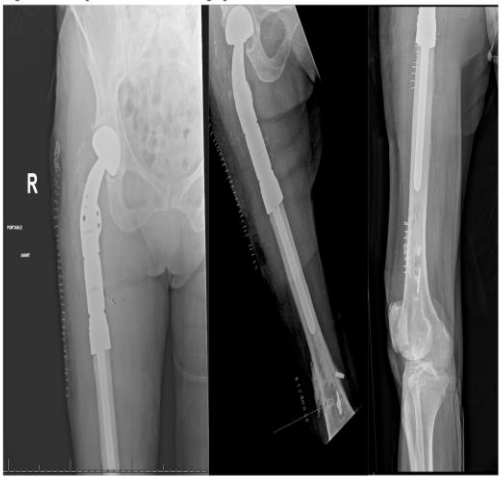


Figure 2: Patient Demographics and Cancer History

	Age/Gender	BMI	Diabetes	Active Smoker	Fracture Site	Years Since Prior Procedure	Type of Cancer	Prior Radiation to site
Patient 1	68/M	29.95	No	No	Right subtrochanteric femur	7.25	Multiple myeloma	Yes
Patient 2	59/M	35.28	No	No	Left subtrochanteric Femur	Unknown	Metastatic lung cancer	Yes
Patient 3	63/M	17.94	Yes	No	Right subtrochanteric femur	3.5	Multiple myeloma	Yes
Patient 4	71/F	24.02	Yes	No	Right subtrochanteric femur	2	Metastatic breast cancer	Yes
Patient 5	71/F	19.40	Yes	No	Right subtrochanteric femur	1.5	Metastatic breast cancer	Yes

Figure 3: Postoperative fixation radiographs from Patient 5:



(P 486) PRIMARY HIGH-GRADE SARCOMA OF THE SPINE: EXPERIENCE WITH 11 CASES

Ryunosuke Fukushi, MD[1]; Makoto Emori, MD PhD[1]; Hiroyuki Tsuchie, MD[2]; Naoya Nakahashi, MD[1]; Junya Shimizu, M.D.[1]; Yasutaka Murahashi, M.D.[1]; Hiroyuki Nagasawa, MD[3]; Naohisa Miyakoshi, Prof.[3]; Toshihiko Yamashita, President[1], (1) Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan, (2) Akita University Graduate School Of Medicine, Akita, Akita, Japan, (3) Akita University Graduate School of Medicine, Akita, Akita, Japan

Objective: Spinal sarcoma presents a challenge for treatment. Although resection and reconstruction have been effective in the treatment of primary spinal sarcomas, there are limitations to resection and reconstruction of mobile spines other than the sacral spine, such as preservation of the spinal cord and cauda equina. Therefore, a consistent treatment plan is often impossible. Furthermore, because primary spinal sarcomas are extremely rare, limited

reports are available on their treatment outcomes. In this study, we report our experience with 11 cases of primary spinal sarcomas and compare the clinical outcomes of the resected and reconstructed patients (R group) with those of the unresected patients (N group).

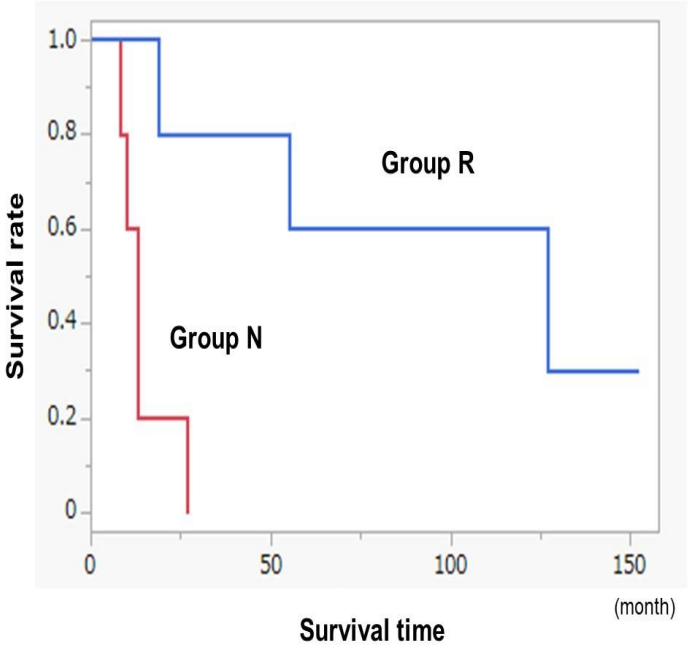
Methods: In total, 11 patients with primary spine sarcoma treated during the 10-year period from 2010 to 2020 were assessed. The R group consisted of 6 patients (three male and three female) while the N group had 5 (two male and three female). The mean age was 51.8 (15–71) years in the R group and 65.6 (36–91) years in the N group. Group R consisted of two cases of Ewing's sarcoma (Th6, S2), two cases of leiomyosarcoma (L4, L4), one case of chondrosarcoma (C6), and one case of undifferentiated pleomorphic sarcoma (Th10). Whereas Group N consisted of two cases of osteosarcoma (Th9, Th11/12), one case of rhabdomyosarcoma (L5), one case of clear cell sarcoma of soft tissue (L5), and one case of undifferentiated pleomorphic sarcoma (L4). We analysed and compared the treatment outcomes, survival, and Activities of Daily Living (ADL) between the two groups. The overall survival (OS) was estimated using Kaplan–Meier plots and the prognostic significance was evaluated using the log-rank test.

Results: The mean tumour diameter at the first visit was 48.3 (24–68) mm in the R group and 47.6 (38–75) mm in the N group. The Weinstein classification was IIA in one case and IIB in five cases in Group R, and IIA in two cases and IIB in three cases in Group N. Of the six patients in Group R, four received chemotherapy, two received radiation therapy, and one received carbon ion radiotherapy. Of the five patients in Group N, three received chemotherapy, two received radiation therapy, and one received proton therapy. Metastases to other organs were observed in 7 patients (63.6%). Recurrence was observed in 2 (33%) patients in the R group. Comparing between the two groups, metastases to other organs were observed in three patients in Group R (50%) and four in Group N (80%). In Group R, two patients (33%) were continuously disease free, one (17%) was alive with disease, two (33%) died of disease (DOD), and one died (17%) of death due to other causes (DOC); while in Group N, all patients were dead, including four (80%) cases of DOD and one (20%) case of DOC. The 5-year OS rates were 60% for Group R and 0 % for Group N and the 5-year OS in Group R was associated with significantly improved survival ($p=0.0055$). Regarding postoperative ADLs, five patients (83%) in Group R and one patient (20%) in Group N were ambulatory.

Conclusion: Despite the differences in patient background and sarcoma status, Group R had a relatively better remission rate, survival, and postoperative ADL than Group N. The results suggest that resection and reconstruction should be the first choice of treatment for primary spinal sarcoma, if indicated, in terms of remission rate, survival, and postoperative ADL. However, the degree of tumour invasion and the patient's general condition must be considered.

		Group R	Group N
Patient background			
Number of patients		6	5
Sex		3･3	2･3
Age		51.8(15-71)	65.6(36-91)
Tumor type	Ewing sarcoma	2	
	Leiomyosarcoma	2	
	Chondrosarcoma	1	
	Undifferentiated pleomorohic sarcom	1	1
	Osteosarcoma		2
	Rhabdomyosarcoma		1
	Clear cell sarcoma of soft tissue		1
Location of Tumor		C:1, T:2, L:2, S:1 (*1)	T:2, L:3
Tumour diameter at initial diagnosis	(Centimeter)	48.3(24-68)	47.6(38-75)
Weinstein Classification	ⅡA	1	2
	ⅡB	5	3
Treatment plan			
Chemotherapy	+	4	3
	-	2	2
Radiation therapy	+	2	2
	-	4	3
Carbon ion radiotherapy	+	1	0
	-	5	5
Proton therapy	+	0	1
	-	6	4
Consequence			
Recurrence	+	2	-
	-	4	-
Metastasis to other organs	+	3	4
	-	3	1
Survival time	(month)	69.8(11-152)	14.2(8-27)
Disease-free survival	(month)	39.8(11-152)	-
5-year Overall survival		60%	0%
Outcome	CDF (*2)	2(33%)	0(0%)
	AWD (*3)	1(17%)	0(0%)
	Dead	3(50%)	5(100%)
Cause of death	DOD (*4)	2	4
	DOC (*5)	1	1
ADL (*6)	Walkable	5(83%)	1(20%)
	Unable to walk	1(17%)	4(80%)

(*1) C:cervical T:thoracic L:lumbar S:sacrum
(*2) continuous disease free
(*3) alive with disease
(*4) dead of disease
(*5) death due to other cause
(*6) activities of Daily Living



(P 487) EN BLOC RESECTION FOR SPINAL TUMORS: A SINGLE CENTER EXPERIENCE OF 113 CONSECUTIVE CASES

Ran Wei, MD; Yiyang Yu, MD; Yi Yang, MD; Tao Ji, MD; Wei Guo, N/A, M.D.; Xiaodong Tang, M.D., Peking University People's Hospital, Beijing, Beijing, China (People's Republic)

Objective: This study aims to assess the clinical outcome of patients with spinal tumors who received en bloc resection and to provide theoretical basis for developing novel surgical technique in en bloc resection for spinal tumor.

Methods: We retrospectively reviewed the clinical and follow-up data of patients who received en bloc resection for spinal tumors in our center from Jan. 2007 to Dec. 2021. The surgical approach of en bloc resection for spinal tumors was determined according to the surgical classification for spinal tumors proposed by Boriani. The general condition, surgical treatment, peri- and post-operative complication, and survival of patients were retrospectively analyzed.

Results: 113 patients were enrolled in this study. There were 68 males and 45 females with an average age of 38.7±16.7 years. Primary malignant spinal tumors, primary aggressive spinal tumors and solitary metastatic spinal tumors were diagnosed in 55, 27 and 31 patients, respectively. All surgeries accomplished uneventfully except one patient died during surgery owing to the haemorrhagic shock caused by injury of major blood vessel. Mean operative time and volume of intra-operative haemorrhage was 517.6±267.4 min and 3802.7±3039.4 mL, respectively. R0 resection was achieved in 48 patients. Three patients died in peri-operative period. Peri-operative complications were identified in 44 patients (38.9%). 109 patients were followed up with a mean follow-up time of 39.4±35.2 months. 27 patients died during follow-up period and the mean post-operative overall survival (OS) was 115.4 (95% CI 97.9-133.0) months. In patients with primary malignant spinal tumors, the OS of patients who underwent R2 resection was significantly poorer than that of patients who underwent R0/R1 resection (P=0.024); while in patients with metastatic tumors, no difference of OS was observed in patients with different surgical margins (P=0.61). There were 29 recurrences, and the mean recurrence-free survival (RFS) was 105.8 (95% CI 87.5-124.1) months. In patients with primary malignant and aggressive spinal tumors, the RFS of patients underwent R2 resection was significantly lower than that of patients underwent R0/R1 resection (P=0.008, P=0.027); but in patients with metastatic spinal tumors, no significant difference of RFS was found in patients with different surgical margin (P=0.71).

Conclusion: Although en bloc resection for spinal tumors indicates complicated surgical procedure and high morbidity, it can significantly improve the OS of patients with primary malignant spinal tumors and the local control of primary malignant and aggressive spinal tumors. However, en bloc resection for spinal tumor showed no improvement in OS and local control in patients with solitary metastatic spinal tumor. It implies that further study should be focused on verifying whether metastatic tumor should be one of the indication of en bloc resections for spinal tumors or not.

(P 488) POSTOPERATIVE DRAINAGE MANAGEMENT AND WOUND COMPLICATIONS FOLLOWING RESECTION OF LOWER LIMB SOFT TISSUE TUMORS

Jens Jakob, MD[1]; Andreas Gerken, MD[2]; Philipp Jawny, MD[3]; Helene Weigl, MD[2]; Franka Menge[4]; Christel Weiß, PhD[2]; Peter Hohenberger, N/A, n/a[4], (1) University Medical Center Mannheim, University of Heidelberg, Mannheim, Baden-Wurttemberg, Germany, (2) University Medical Center Mannheim, Mannheim, Baden-Wurttemberg, Germany, (3) Department of Cardiothoracic Surgery, University Hospital Augsburg, Augsburg, Bayern, Germany, (4) Sarcoma Unit, Department of Surgery and Mannheim Cancer Center, University Medical Center Mannheim, University of Heidelberg, Mannheim,Germany, Mannheim, Baden-Wurttemberg, Germany

Objective: Postoperative wound complications are common in sarcoma surgery. Postoperative drainage therapy may indicate postoperative bleeding and support wound healing by avoiding seroma but may also cause complications such as secondary infection. Prolonged drainage therapy may delay adjuvant treatment and rehabilitative measures. The aim of this study was to evaluate the incidence of postoperative wound complications and delayed or prolonged drainage treatment in lower limb tumors.

Methods: A retrospective analysis of 80 patients who underwent primary resection of lower extremity soft tissue tumors was performed. Wound complications (e.g. infection), known risk factors for wound complications (e.g. large tumor size) as well as postoperative drainage management were documented. A classification of postoperative wound complications was developed. The classification took into account prolonged drainage management.

Results: The proposed classification of postoperative wound complications taking into account draiange management is depicted in table 1. A so-called regular postoperative course (grade 0) was documened in 33% of all patients. Minor wound complications or delayed drainage removal (grade A) occured in 15%. Major wound complications or prolonged drainge therapy (grade B) and reoperations (grade C) were documented in 39% and 13%.Tumor size ($p = 0.0004$), proximal tumor location ($p = 0.0484$), and tumor depth ($p = 0.0138$) were identified as risk factors for complex postoperative courses (grade B or C). Drainage volume on postoperative day 4 was a suitable predictor for complex courses (cutoff of 70 ml/d).

Conclusion: We confirm a high rate of postoperative complications after surgery for lower extremity tumors. We propose to include prolonged drainage management into aclassification of postoperative wound complications to take into account the increased need for inpatient as well as outpatient care for the affected patients including the delay of adjuvant treatment and rehabilitative measures. We propose drainage volume on day 4 as a predictor for a complex postoperative course.

Grade	Drainage removal	Wound complications	Description
0	in time	No complications	Timely drainage removal within 4 days and no wound complication
A	delayed	Minor complications*	Delayed wound drainage≥5th postoperative day and/or wound complications without need for intervention
B	prolonged	Major complications**	Prolonged wound drainage≥10th postoperative day and/or wound complications neces□sitating intervention (e.g. puncture/drainage of seroma, readmission for wound therapy or intravenous antibiotic therapy)
C		Reoperation***	Need for reoperation (e.g., for bleeding or wound breakdown

* minor: CTCAE Grade I, Clavien Dindo≤grade II; **major: CTCAE grade II, Clavien Dindo≤grade IIIa; ***; reoperation: CTCAE grade III, Clavien Dindo≤grade IIIb

(P 489) SIMULTANEOUS VERSUS DELAYED FLAP RECONSTRUCTION FOLLOWING SOFT TISSUE SARCOMA RESECTION

Pearce B. Haldeman, B.S.[1]; Liane Chun, MD[2]; Christopher Reid, MD[2]; James H. Flint, MD, FACS[2]; Frank Chiarappa, MD[2]; Conner D. Trimm, B.S.[3], (1) University of California San Diego School of Medicine, La Jolla, CA, (2) University of California San Diego, San Diego, CA, (3) University of California San Diego School of Medicine, San Diego, CA

Objective: The morbidity associated with soft tissue sarcomas (STS) treatment is significant considering the preferred surgical option is a limb sparing wide local excision. Accordingly, there are well-documented complications associated with wound healing in this population. To abate these complications, nuanced reconstruction methods including the use of muscle flaps may be utilized as a means to protect the surgical site and promote wound healing. Though, reconstruction methods are not without cost given they are associated with longer hospital stays and may necessitate multiple surgeries in the form of a planned reconstruction following completion of the original procedure. This retrospective study serves to compare surgical and oncologic outcomes between patients receiving simultaneous or delayed flap reconstruction following STS resection.

Methods: 37 patients who underwent flap reconstruction following resection of a primary STS at a tertiary referral center for musculoskeletal tumors from 2019 to 2022 were retrospectively evaluated. Patients who received primary wound closure but subsequently required reconstruction due to complications were excluded (n=3). Of the 34 remaining patients, 24 (71%) received simultaneous reconstruction during the index surgery and 10 (29%) underwent planned delayed reconstruction. Preoperative patient specific metrics, tumor characteristics, surgical, and patient outcomes were collected and are organized in Table 1. The greatest

dimension upon radiographic and pathologic measurement was the recorded size. For patients who received delayed reconstruction, the intraoperative and surgical hospitalization metrics for both surgeries were summed and used for data analysis. Significance of differences in means was determined using independent T-tests. Significance of differences between frequencies was determined using chi-square or Fisher's exact tests.

Results: Mean time between resection and planned reconstruction for those undergoing delayed reconstruction was 9.7 ± 2.7 days. Mean follow-up was 15.91 ± 10 and 17.17 ± 14 months in the simultaneous and delayed cohorts, respectively. Age, sex, BMI, ASA class, tumor grades, presence of metastases at time of surgery, EBL, and OR time were similar amongst both groups (P-values=0.328, 0.575,0.919, 0.069, 0.914, 0.162, 0.184, 0.409). More patients (P-value=0.021) in the simultaneous reconstruction group received chemotherapy in the neoadjuvant setting than did the delayed group. There were no statistical differences between other neoadjuvant or adjuvant therapies, including radiation. Hospital length of stay (HLOS) and complications during operative hospitalization were similar amongst both groups (P-values=0.555, 0.760). Though surgical margins were more often negative in the delayed group (P-value=0.043), the rate of unplanned positive surgical margins was no different between groups (P-value=0.328). The overall postoperative complication, reoperation, and 90-day unplanned readmission rates were equivalent in both groups (P-values=0.935, 0.336, 0.857). The disease specific survival rate at last follow-up was similar in both groups (P-value=0.328) (Table 1).

Conclusion: This retrospective chart review study evaluated surgical and oncologic outcomes for patients undergoing simultaneous or delayed flap reconstruction following STS resection. These data indicate the populations were similar regarding their demographic and comorbid characteristics. It is important to note that more patients in the simultaneous coverage group were receiving chemotherapy and 2 patients had metastases prior to resection, possibly indicating a worse burden of disease amongst that group. Surgical outcomes, complications, readmissions, reoperations, unplanned positive resection margin and disease specific survival were equivalent in both groups suggesting either method can be utilized safely. Patients with planned positive margins had unresectable disease with intraoperative brachytherapy (1) or metastatic disease (2). Each patient undergoing treatment of STS was managed by a multidisciplinary team, and surgical decision making was performed based on patient and tumor characteristics. The advantage of simultaneous reconstruction is that it can be accomplished during a single surgery and anesthetic episode. Alternatively, a critical feature that favors a delayed reconstruction is the ability to assure positive margins prior to final closure. This is valuable in the setting of certain histologic tumor types, such high-grade myxofibrosarcoma, or if the tumor is adjacent to a critical structure and obtaining a negative margin is less certain. In conclusion, this study finds that either simultaneous or delayed flap reconstruction following STS resection can be appropriately implemented in the setting of shared decision making based on a patient's specific goals of care, tumor characteristics, histology, and tumor location. Future research will focus on mitigating limitations of this study such as improving sample sizes, collecting patient reported outcome data, increasing follow-up length and performing propensity matching to decrease error due to confounders.

	Simultaneous (n=24)	Delayed (n=10)	P-value
Age	62.125 ± 18.1	54.6 ± 20.4	0.328
Sex			0.575
Male	7 (29%)	4 (40%)	
Female	17 (71%)	6 (60%)	
BMI	27.75 ± 5.9	27.56 ± 4.8	0.919
ASA Class	2.9 ± 0.45	2.5 ± 0.53	0.069
Tumor Histology			
Liposarcoma	4 (17%)	0 (0%)	**0.043**
Synovial sarcoma	4 (17%)	1 (10%)	0.604
Myxofibrosarcoma	8 (33%)	2 (20%)	0.431
Pleomorphic sarcoma	8 (33%)	3 (30%)	0.857
Dematofibrosarcoma protuberans	0 (0%)	4 (40%)	**0.037**
Tumor Location			0.670
Trunk	3 (10%)	2 (20%)	
Proximal Upper Extremity	2 (8%)	1 (10%)	
Distal Upper Extremity	1 (4%)	0 (0%)	
Proximal Lower Extremity	14 (60%)	5 (50%)	
Distal Lower Extremity	4 (17%)	2 (20%)	
Tumor Federation Nationale de Centres de Lutte Contre le Cancer (FNCLCC) Grade	2.3 ± 0.76	2.3 ± 0.82	0.914
1	4 (17%)	2 (20%)	
2	8 (33%)	3 (30%)	
3	12 (50%)	5 (50%)	
Radiographic Tumor Size (cm)	8.66 ± 5.2	7.24 ± 3.1	0.346
Pathologic Tumor Size (cm)	9.05 ± 5.7	9.38 ± 4.7	0.870
Known Metastasis at Surgery			0.162
Yes	2 (8%)	0 (0%)	
No	22 (92%)	10 (100%)	
Operating Room (OR) Time (minutes)	696.1 ± 424	541.9 ± 230	0.184
Estimated Blood Loss (EBL) (mL)	332.9 ± 839	180.5 ± 194	0.409
Intraoperative Complications			0.328
Yes	1 (4%)	0 (0%)	
No	23 (96%)	10 (100%)	
Neoadjuvant Radiation			0.258
Yes	15 (63%)	4 (40%)	
No	9 (37%)	6 (60%)	
Adjuvant Radiation			0.608
Yes	5 (21%)	3 (30%)	
No	19 (79%)	7 (70%)	
Neoadjuvant Chemotherapy			**0.022**
Yes	5 (21%)	0 (0%)	
No	19 (79%)	10 (100%)	
Adjuvant Chemotherapy			0.417
Yes	5 (21%)	1 (10%)	
No	19 (79%)	9 (90%)	
Hospital Length of Stay (HSOL) (days)	5.54 ± 6.8	6.9 ± 5.6	0.555
Complications During Operative Hospitalization			0.760
Yes	6 (25%)	2 (20%)	
No	18 (75%)	8 (80%)	
Pathologic Resection Margin			
Negative	20 (83%)	10 (100%)	**0.043**
Planned Positive	3 (13%)	0 (0%)	0.082
Unplanned Positive	1 (4%)	0 (0%)	0.328
Mean Follow-up (months)	15.91 ± 10	17.17 ± 14.7	0.808
Number of Postoperative Complications	10 (42%)	4 (40%)	0.935
Wound Complication (delayed healing, dehiscence)	8 (33%)	4 (40%)	
Infection (cellulitis, deep surgical site infection, infected hardware)	6 (25%)	2 (20%)	
Reoperations Performed	8 (33%)	2 (20%)	0.336
Unplanned Readmissions within 90 days postop	8 (33%)	3 (30%)	0.857
Disease Specific Survival Rate	96%	100%	0.328

(P 490) **TOPICAL ANTIBIOTICS DECREASE INFECTIONS IN EXTREMITY SARCOMA SURGERY**

Pearce B. Haldeman, B.S.[1]; Conner D. Trimm, B.S.[2]; Christopher Reid, MD[3]; James H. Flint, MD, FACS[3]; Frank Chiarappa, MD[3], (1) University of California San Diego School of Medicine, La Jolla, CA, (2) University of California San Diego School of Medicine, San Diego, CA, (3) University of California San Diego, San Diego, CA

Objective: Surgical treatment of sarcoma often involves large complex dissections necessitating complicated closure techniques, poorly vascularized wound beds, long operative times, and treatment of compromised hosts due to radiation and/or chemotherapy. All of which increase the risk of postoperative surgical site infections. Antibiotics in the form of powders, dissolvable calcium sulfate or polymethylmethacralate (PMMA) beads, or as a tissue/arthroplasty spacer have been utilized in other orthopedic surgery specialties to mitigate or treat perioperative infections. The efficacy of these antibiotic adjuvants has not been evaluated in sarcoma surgery. This study serves to compare outcomes of patients who received topical antibiotics, in the form of vancomycin powder, with those who did not while undergoing resection of extremity sarcomas.

Methods: 81 patients who underwent resection of primary extremity sarcoma at a tertiary referral center for musculoskeletal tumors from 2019 to 2022 were retrospectively evaluated. 24 patients (30%) were found to have received topical vancomycin powder just prior to wound closure, while 57 patients (70%) had not. Administration of topical antibiotic powder was at the discretion of the operating surgeon(s) and generally utilized in high-risk wounds as determined by the operating team. Patient specific metrics, tumor characteristics, and surgical and patient outcomes were collected for all patients and are organized in Table 1. Tumor grade is based on

the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) system. The greatest dimension upon radiographic and pathologic measurement was the recorded size. Significance of differences in means was determined using independent T-tests. Significance of differences between frequencies was determined using chi-square or Fisher's exact tests.

Results: Mean follow-up was 16.9 ± 9.8 months in the topical antibiotic group and 20.1 ± 12.4 in the control group. No patients experienced systemic side effects from topical vancomycin use. Age, sex, body mass index (BMI), American Society of Anesthesiologists (ASA) Class, tumor location, tumor grade, and presence of metastases at time of surgery were similar amongst both groups. 46% of patients who received antibiotics had sarcoma originating from bone compared to only 12% of patients who did not (P-value=0.006). Tumor histology differed between the two groups with more patients in the no antibiotic group having leiomyosarcoma (P-value=0.024). An equivalent number of patients in both groups were receiving either radiation or chemotherapy in the adjuvant or neoadjuvant setting. The radiographic and pathologic tumor size was greater in the antibiotic group (P-values=0.03, 0.032); also estimated blood loss (EBL), operating room (OR) time and hospital length of stay were greater in the antibiotic group (P-values=0.019, 0.001, 0.006). The number of positive surgical pathology margins and intraoperative and postoperative hospitalization complications were equivalent in both groups. Patients receiving topical antibiotic powder experienced significantly less postoperative infections (P-value=0.035). Rates of non-infectious postoperative complications, such as deep vein thrombosis (n=2), sterile delayed wound healing (n=2), inadequate pain control (n=1), and pathologic fracture (n=1), were similar in both groups. The 90-day postoperative unplanned readmission rate and overall survival rate were similar in both groups.

Conclusion: This study compared the surgical, oncologic, and infectious outcomes of patients receiving intraoperative topical antibiotics with those who did not during sarcoma resection over an approximately 18-month period. These data indicate that both groups were similar in their demographic and comorbid characteristics, though it is apparent that the group of patients who received intraoperative topical antibiotics were suffering from increased disease burden as evidenced by their larger tumor size, longer operating time, and longer operative hospitalization time. Despite this, topical vancomycin powder significantly decreased the rate of postoperative infections. Of note, use of antibiotics was under the discretion of the surgeon and generally used for higher-risk cases, which presents a limitation of the study. These preliminary results demonstrate a role for topical antibiotics in the prevention of postoperative infections in extremity sarcoma surgery. Future research will need to focus on improving sample sizes, achieving better matching of cohorts, performing multivariate analysis to better control for confounders, and ultimately performing a randomized trial.

	No topical antibiotic (n=57)	Topical antibiotic (n=24)	P-value
Age	58 ± 18	53 ± 21	0.258
Sex			0.275
Male	28 (49%)	15 (62.5%)	
Female	29 (51%)	9 (37.5%)	
BMI	28.2 ± 6.6	30 ± 5.8	0.234
ASA Class	2.6 ± 0.6	2.7 ± 0.5	0.542
Tumor Origin			**0.006**
Bone	7 (12%)	11 (46%)	
Soft Tissue	50 (88%)	13 (54%)	
Tumor Histology			
Liposarcoma	6 (10%)	4 (17%)	0.489
Synovial Sarcoma	2 (4%)	0 (0%)	0.159
Myxofibrosarcoma	16 (28%)	5 (20%)	0.489
Pleomorphic Sarcoma	12 (21%)	2 (8%)	0.114
Dematofibrosarcoma Protuberans	3 (5%)	1 (4%)	0.832
Leiomyosarcoma	5 (9%)	0 (0%)	**0.024**
Chondrosarcoma	1 (2%)	3 (13%)	0.143
Osteosarcoma	5 (9%)	6 (25%)	0.107
Other	7 (12%)	3 (13%)	0.979
Tumor Location			0.312
Proximal Upper Extremity	8 (14%)	1 (4%)	
Distal Upper Extremity	3 (5%)	0 (0%)	
Proximal Lower Extremity	39 (69%)	17 (71%)	
Distal Lower Extremity	7 (12%)	6 (25%)	
Tumor Grade	2.5 ± 0.7	2.4 ± 0.8	0.757
1	6 (11%)	3 (13%)	
2	15 (26%)	4 (17%)	
3	36 (63%)	17 (70%)	
Radiographic Tumor Size (cm)	7.61 ± 5.15	11.64 ± 7.79	**0.030**
Pathologic Tumor Size (cm)	8.68 ± 6.83	12.55 ± 7.31	**0.032**
Known Metastasis at Surgery			0.599
Yes	4 (7%)	1 (4%)	
No	53 (93%)	23 (96%)	
OR Time (minutes)	475 ± 322	783 ± 381	**0.001**
Estimated Blood Loss (mL)	180 ± 544	442 ± 399	**0.019**
Intraoperative Complications			0.083
Yes	3 (5%)	0 (0%)	
No	54 (95%)	24 (100%)	
Neoadjuvant Radiation			0.601
Yes	25 (44%)	9 (38%)	
No	32 (56%)	15 (62%)	
Adjuvant Radiation			0.646
Yes	12 (21%)	4 (17%)	
No	45 (79%)	20 (83%)	
Neoadjuvant Chemotherapy			0.924
Yes	9 (16%)	4 (17%)	
No	48 (84%)	20 (83%)	
Adjuvant Chemotherapy			0.680
Yes	14 (25%)	7 (29%)	
No	43 (75%)	17 (71%)	
Hospital Length of Stay (days)	4 ± 5.5	9 ± 7.6	**0.006**
Complications During Operative Hospitalization			0.288
Yes	8 (14%)	6 (25%)	
No	49 (86%)	18 (75%)	
Pathologic Resection Margin			0.832
Positive	3 (5%)	1 (4%)	
Negative	54 (95%)	23 (96%)	
Mean Follow-up (months)	20.1 ± 12.4	16.9 ± 9.8	0.230
Number of Postoperative Infections	22 (38%)	4 (17%)	**0.035**
Minor Infections (non-operative management)	6 (10%)	0 (0%)	**0.013**
Major Infections (requiring operative management)	16 (28%)	4 (17%)	0.251
Number of Non-Infectious Postoperative Complications	3 (5%)	3 (13%)	0.343
Number of Unplanned Readmissions within 90 days postoperatively	19 (33%)	7 (29%)	0.716
Overall Survival	91%	96%	0.416

(P 491) **COMPARISON OF FIXATION TECHNIQUES FOR METASTATIC DISEASE OF THE HUMERUS**

Mikaela Sullivan, MD; Alexandra Arguello, MD; Peter S. Rose, MD; Matthew T. Houdek, MD, Mayo Clinic, Rochester, MN

Objective: The humerus is a common location for metastatic disease, and numerous options are available for fixation in patients with impending or pathologic fracture. Both intramedullary fixation and cement augmented plate osteosynthesis have been shown successful in the management of humeral metastases, however these techniques have not been compared. The aim of this study is to compare functional outcomes and complications between plate and intramedullary nail constructs in patients undergoing fixation for humeral metastases.

Methods: A total of 67 cases in 64 (38 male, 26 female) patients undergoing fixation of the humerus for metastatic disease were retrospectively reviewed. Mean age was 63 years, and most common diagnoses included multiple myeloma (39%), renal cell carcinoma (22%), breast carcinoma (8%), and lung carcinoma (8%). Fixation included 25 antegrade humeral nails and 42 plates with cement augmentation. Pathologic fracture was the indication in 54% of cases and prophylactic fixation in 46%. Lesions were located in the proximal humerus in 42%, midshaft humerus in 46%, and distal humerus in 12%. Average follow up was 1.5±1.9 years, and 34% are still living.

Results: Patients with plate fixation had significantly higher MSTS scores (76% vs. 66%, p=0.004) and better post-operative forward elevation (123° vs. 90°, p=0.008) compared to those with nail fixation. Complication rates included 6% fracture, 3% implant failure, 6% continued pain, and 5% reoperation, with no differences between constructs. Satisfaction rates were also similar at final follow-up, including 90% for nail fixation and 94% for plate fixation

(p=0.59). There was no significant difference between groups in terms of age, sex, BMI, follow up, proportion of pathologic fracture, or diagnosis, though there was a significantly higher proportion of distal humerus lesions in the plate fixation group (19% vs. 0%, p=0.021).

Conclusion: In this series, patients with metastatic disease of the humerus had improved post-operative range of motion as well as higher functional outcome scores when undergoing fixation with a plate construct compared to a nail construct. No difference was observed in terms of complications or patient satisfaction. For patients with metastatic disease of the humerus requiring pathologic or prophylactic fixation, plate constructs may be preferred when patient factors are amenable.

(P 492) **BALANCE AND GAIT OUTCOMES AFTER 1-YEAR POST-OPERATIVE HEMIPELVECTOMY SURGERY**

Sherron Furtado, PhD[1]; Jinxuan Zhao, MSc[2]; Stephen JG Taylor, MSc PhD[3]; Catherine Pendegrass, n/a[4]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[5], (1) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom, (2) University College London, London, England, United Kingdom, (3) University College London, Stanmore, England, United Kingdom, (4) University College London, Greater London, England, United Kingdom, (5) Royal National Orthopaedic Hospital, London, England, United Kingdom

Objective: Currently, the focus when considering post-operative functional assessment in sarcoma patients is to measure their disability, while balance (postural stability(issues and their investigation is lacking in most sarcoma research. Static balance (during standing) has been investigated in two pilot studies (De Visser 2001, Furtado et al 2020) respectively, but there are no studies that look at dynamic balance (during walking) even though people with musculoskeletal cancers present with significant balance problems and falls in the long-term. This study aimed to investigate the feasibility of assessing dynamic balance after hemipelvectomy surgery for a sarcoma.

Methods: This is a retrospective cross-sectional pilot and feasibility study (Ethics approval: 21/WA/0027) analysing existing datasets from the Gait Real-time Analysis Interactive Laboratory (GRAIL) database at RNOH for 10 sarcoma patients who underwent hemipelvectomy one year ago and 10 healthy participants (controls) . The margin of stability (MOS) was used to quantify dynamic balance ability in all participants during treadmill walking. MOS in the anterior, posterior, lateral and medial direction, stride length and stride width of the patients and controls at heel contact or during the swing phase were compared using the Mann-Whitney U-test.

Results: 10 patients (nine males and 1 female) with pelvic sarcomas of mean age, weight and height of 41(±13) years, 78.4(±16.9)kg, 1.77(±0.10)m were enrolled into this study. No statistically significant differences were seen in patient MOS between the operated and non-operated side during the swing phase ($p>0.05$), but it was found that the patient's stride length was usually longer on the operated side than on the non-operated side ($p< 0.05$*). Furthermore, it found that patients had significantly smaller values of posterior MOS during the swing phase ($p< 0.05$*) and smaller values of medial MOS during heel touch compared to controls ($p< 0.05$*) (Fig. 1).

Conclusion: This is the first study demonstrating that it is feasible to quantify dynamic balance in the sarcoma population. It is pertinent to note that, MOS identified struggling the patients with balance issues during walking. However, no differences were found between the balance of the operated and non-operated side of the leg during the swing phase and the stride length of the operated leg was longer than the non-operated leg in patients after hemipelvectomy. Encouraging balance training using physiotherapy and rehabilitation strategies when walking in the posterior and medial directions, may help to improve overall body balance.

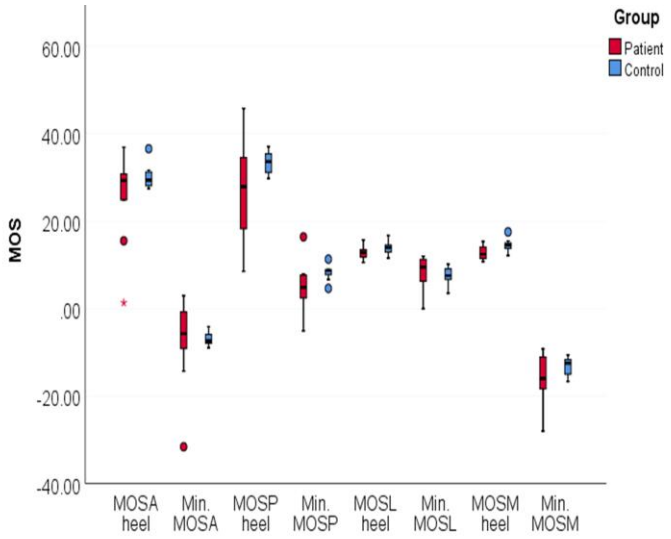


Fig. 1: Patients had significantly smaller values of posterior MOS during the swing phase (Z=-2.42, p=0.016*) and smaller values of medial MOS during heel touch compared to controls (Z=-2.50, p=0.013*). However, no significant differences were found between the patient and control groups in other directions across the gait cycle.

(P 493) GAIT KINEMATICS OF SARCOMA PATIENTS AT 1 YEAR POST-OPERATIVE INTERNAL HEMIPELVECTOMY SURGERY: A RETROSPECTIVE STUDY.

Sherron Furtado, PhD[1]; shruti Modi, MSc[2]; Stephen JG Taylor, MSc PhD[3]; Catherine Pendegrass, n/a[2]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[4], (1) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom, (2) University College London, Greater London, England, United Kingdom, (3) University College London, Stanmore, England, United Kingdom, (4) Royal National Orthopaedic Hospital, London, England, United Kingdom

Objective: Gait deviations are commonly seen in clinics in sarcoma patients who undergo internal hemipelvectomy (IH). To assess the effectiveness of the surgery and rehabilitation, the objective examination of gait deviations is crucial, but lacking. The Gait Profile Score (GPS) and the Gait Variable Score (GVS) were developed in order to summarize the kinematic gait data. The main purpose of this study was to investigate the feasibility of capturing these scores in this patient population and understand its clinical value.

Methods: This is a retrospective cross-sectional pilot and feasibility study (Ethics approval: 21/WA/0027) of 10 patients who underwent IH for a sarcoma diagnosis one year ago. Data

from Gait Real-time Analysis Interactive Laboratory (GRAIL) was analysed to measure GPS, GVS and gait speed. A normative GPS score was calculated using the kinematic data of controls, whereas individual scores were quantified for patients. GPS and gait speed were compared between patients and controls using Mann-Whitney U test. Spearman's correlations were used to study relationships between GPS and gait speed and GPS and the disease-specific 'Toronto Extremity Salvage Score (TESS)' scores.

Results: The mean age of our patients were 41.5±11.77 years. Median GPS values for the patients and healthy individuals were 10.36˚ (8.15˚ to 14.62˚) and 1.22˚ respectively (Fig. 1) and these differences were statistically significant (p< 0.01*), indicating gait abnormalities can be identified using the GPS, a single score. The GVS further helped this understanding by emphasising that the hip, pelvis, knee, and ankle were compensating. Gait speed was also found to significantly affected in patients [0.70 m/s (0.62 to 0.91 m/s)] compared to controls [1.2 m/s] (p< 0.05*). Although correlations between the GPS and gait speed and GPS and TESS were not found to be statistically significant (r= -0.465, p= 0.176), the negative correlations were clinically sensible.

Conclusion: This is the first study showing that GPS and GVS is feasible to capture in patients who underwent IH for a sarcoma. GPS identified patients struggling with gait abnormalities, whereas GVS identified compensations during gait. These objective scores can guide rehabilitation assessments and management in busy clinics. GPS and TESS scores can be used in combination to provide clinicians with objective as well as subjective prognoses of a patient and further studies with a larger sample size will be needed to confirm the usefulness and clinical applications of GPS and GVS.

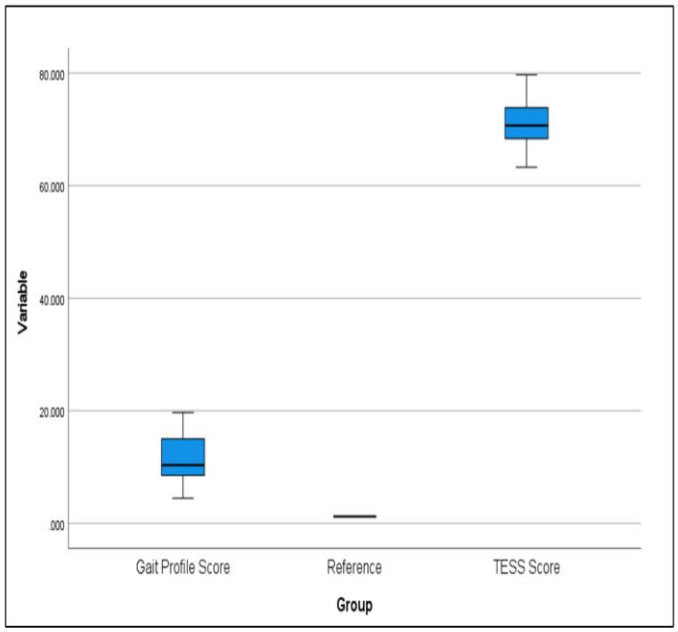


Figure 1: Descriptive statistics for the GPS, Reference (GPS) and TESS score

(P 494) WHAT IS THE EXPERIENCE OF THOSE WITH CANCER-RELATED REDUCED QUALITY OF LIFE AND ASSOCIATED PHYSICAL DISABILITIES AFTER UPPER LIMB SARCOMA TREATMENT IN ENGLAND? A NATIONAL STUDY OF OUTCOMES.

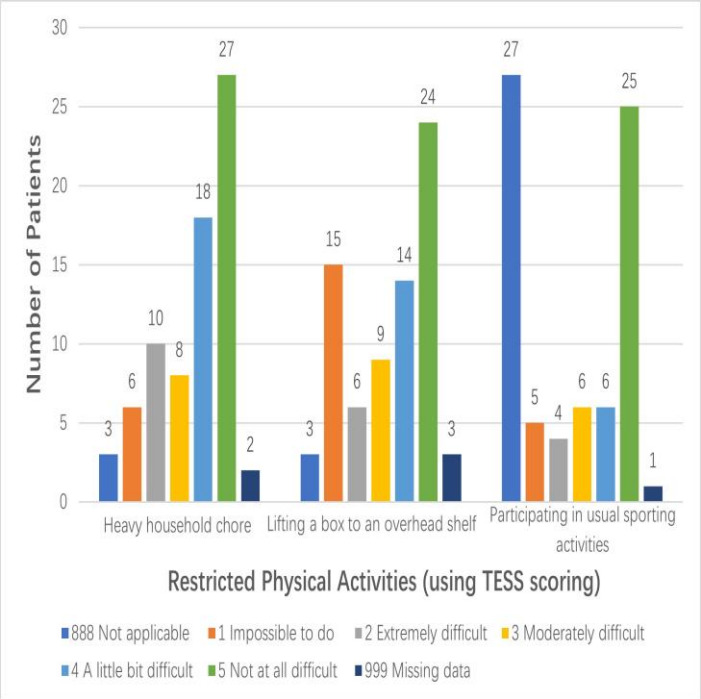
Sherron Furtado, PhD[1]; Yiwen Yan, MSc[2]; Catherine Pendegrass, n/a[2]; Stephen JG Taylor, MSc PhD[3]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[4], (1) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom, (2) University College London, Greater London, England, United Kingdom, (3) University College London, Stanmore, England, United Kingdom, (4) Royal National Orthopaedic Hospital, London, England, United Kingdom

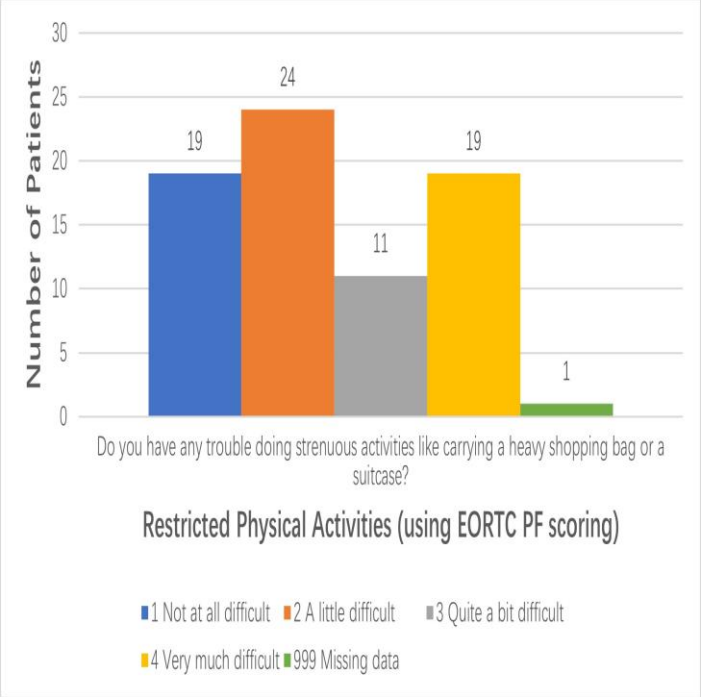
Objective: Significant functional disability and reduced Quality of Life (QoL) are common following treatment for upper limb sarcoma. This study aimed to explore the current extent of physical function and QoL for upper extremity sarcoma patients in England to enhance rehabilitation strategies.

Methods: A retrospective analysis of the upper extremity datasets from the Sarcoma Assessment Measure (SAM) study was conducted. SAM is a national study led by University College London Hospital (UCLH) and funded by Sarcoma UK; aiming at developing a sarcoma-specific outcome measure.

Results: Of 74 patients identified with mean age=62.34, female/male=40/34, 63 were diagnosed with chondrosarcoma and 16 with osteosarcoma. 93% underwent surgery, of which 9% had an amputation. Datasets showed that participants reported high medians of Toronto Extremity Salvage Score (TESS) and European Organisation for Research and Treatment of Cancer (EORTC) QoL Questionnaire (QLQ-C30) (TESS=91.95, EORTC Physical=86.67, Role/ Emotional/Cognitive=83.33, Social=100), indicating good overall function and QoL. However, sub-analysis revealed that activities of daily living (ADLs) were majorly restricted including: heavy household chores, overhead lifting of boxes, participating in usual sporting activities, and strenuous activities. Amputation led to reduced functional outcomes and QoL ($p=0.002^*/0.012^*$) compared to other surgeries. Gender, sarcoma type, combined site tumour, marital status, and ethnicity ($p< 0.05^*$) showed relevance, and functional outcomes were also found to have a strong correlation with QoL ($p< 0.001^*$).

Conclusion: While people treated for upper extremity sarcoma in England show high levels of physical function and QoL experience, it remains important to recognise patients' needs and provide holistic post-treatment support. Patient, tumour, and treatment-related factors all warrant focused attention.


Number of Patients
30
25
20
15
10
5
0
3
6
10
8
18
27
2
3
15
6
9
14
24
3
27
5
4
6
6
25
1
Heavy household chore
Lifting a box to an overhead shelf
Participating in usual sporting activities
Restricted Physical Activities (using TESS scoring)
888 Not applicable
1 Impossible to do
2 Extremely difficult
3 Moderately difficult
4 A little bit difficult
5 Not at all difficult
999 Missing data


Number of Patients
30
25
20
15
10
5
0
19
24
11
19
1
Do you have any trouble doing strenuous activities like carrying a heavy shopping bag or a suitcase?
Restricted Physical Activities (using EORTC PF scoring)
1 Not at all difficult
2 A little difficult
3 Quite a bit difficult
4 Very much difficult
999 Missing data

(P 495) UNDERSTANDING THE EXPERIENCES OF PATIENTS RETURNING TO ACTIVITIES OF DAILY LIVING AND EXERCISE FOLLOWING LIMB SPARING SURGERY FOR BONE OR SOFT TISSUE SARCOMA AROUND THE KNEE.

Sherron Furtado, PhD[1]; Emily Dore-Smith, MSc[2]; Stephen JG Taylor, MSc PhD[3]; Catherine Pendegrass, n/a[4]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[5], (1) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom, (2) University College London, Hampshire, England, United Kingdom, (3) University College London, Stanmore, England, United Kingdom, (4) University College London, Greater London, England, United Kingdom, (5) Royal National Orthopaedic Hospital, London, England, United Kingdom

Objective: A large proportion of sarcomas occur in the extremities, particularly around the knee joint, presenting numerous physical limitations. There is limited qualitative literature providing insight into patients' lives. This sub-study is part of a larger study, the Stanmore Tumour Outcomes Project, measuring survivorship and rehabilitation outcomes in The London Sarcoma Service. Objective: The objective of this study is to understand the experiences and potential barriers of returning to daily activities and exercise following treatment for knee sarcoma, to pre-operatively guide patient expectations and support rehabilitation.

Methods: Methods: In phase one, a narrative literature review was undertaken on sarcoma survivors, with a focus on knee tumours. This informed phase two, a qualitative study of eight patients at least one-year post limb salvage surgery of the knee. Ethical approval was granted from a National Ethics Committee in the UK (reference 21/WA/0027).

Results: Results: Eight patients with around knee sarcoma were included in the study. Patients received either a distal femoral replacement, proximal tibial replacement or a tibia resection with fibula allograft. Four themes emerged, Navigating activities of daily living, Barriers to exercise, The need for mental health support and Lack of local knowledge. Patients demonstrated resilience returning to life after treatment and expressed both positive and negative aspects of recovery.

Conclusion: Conclusion: Most patients following limb sparing surgery continue to experience not only physical limitations but also long-term effects on mental health. Improved individualised rehabilitation and comprehensive follow up perhaps based on biopsychosocial models, may help to mitigate some of the difficulties faced.

(P 496) WHAT ARE THE EXPERIENCES OF BALANCE, GAIT AND NEURO-MUSCULAR CONTROL OUTCOMES AFTER PROXIMAL FEMORAL REPLACEMENTS FOR MUSCULOSKELETAL TUMOURS?

Sherron Furtado, PhD[1]; Wing Ho, MSc[2]; Stephen JG Taylor, MSc PhD[3]; Catherine Pendegrass, n/a[2]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[4], (1) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom, (2) University College London, Greater London, England, United Kingdom, (3) University College London, Stanmore, England, United Kingdom, (4) Royal National Orthopaedic Hospital, London, England, United Kingdom

Objective: Sarcomas are a rare heterogenous group of cancers, that commonly affects the lower limb. Patients with proximal femoral bone tumours frequently undergo proximal femoral

replacement (PFR) surgery to remove the tumour. However, there is limited research on how PFR influences patients' gait, balance, and neuromuscular function post-operatively. This research includes a literature review and a qualitative study. The aim is to explore: 1. Facilitators and barriers for achieving a good outcome 2. How the outcome in these three aspects affects patients' daily life.

Methods: This study is part of the STOMP study (REC ref: 21/WA/0027) in the RNOH London Sarcoma Service. A narrative literature review that identifies current literature gap, help to guide and inform specific questions for the interview. The qualitative research, in the format of online interviews will focus on the patients' experiences and the factors that affect them. Patients, that finished PFR 1-year and aged> 6, are identified from the database of RNOH with purpose sampling. The qualitative review adapted a Rapid Appraisal method, data analysed by rapid appraisal sheet and the Framework method.

Results: The current literature, across different age and diagnosis, does not provide enough information to determine the impact of PFR on patients' daily lives. Most of the evidence suggested that patients' social life and physical abilities are heavily interrupted due to muscle weakness, fatigue, and stiffness. Seven individuals participated in the qualitative study. They mentioned that they struggle with pain and fatigue. Each participant listed out largely diverse factors. Some developed a fear of falling and cancer that caused maladaptive behaviour. These disabilities affected them psychosocially and lowered their quality of life. All mentioned that the local hospitals did not provide enough and good quality physiotherapy.

Conclusion: Patients' gait, balance, and neuromuscular control were significantly impacted due to muscle stiffness and reduced strength. There are no studies that discusses how PFR impacts patients' daily lives. This emphasises proper screening of patients' symptoms and individual functional limitations. Healthcare providers should provide personised treatment accordingly and not only rely on the outcome measures, but also patients' experiences in daily life. Hospital could consider providing treatment plan to local hospital to ensure better care.

(P 497) STANMORE TUMOUR OUTCOMES PROJECT (STOMP)- USING INTERNATIONAL CLASSIFICATION OF FUNCTIONING, DISABILITY AND HEALTH (ICF) OUTCOMES IN SARCOMA SURVIVORS. and UNDERSTANDING THE EXPERIENCES OF PATIENTS RETURNING TO DAILY ACTIVITES AND EXERCISE FOLLOWING LIMB SPARING SURGERY FOR BONE OR SOFT TISSUE SARCOMA AROUND THE KNEE

Teja Joshi, MSc[1]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[1]; Deborah Eastwood, FRCS[2]; Sherron Furtado, PhD[3], (1) Royal National Orthopaedic Hospital, London, England, United Kingdom, (2) RNOH stanmore, london, England, United Kingdom, (3) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom

Objective: Surgical interventions aimed at managing musculoskeletal tumours, including sarcomas, result in notable physical limitations for patients. The Stanmore OutcoMes Project (SOMP) aims to comprehensively identify the physical, psycho-social, functional limitations experienced by patients who have undergone surgeries for musculoskeletal tumours, including massive endoprosthesis replacements, amputations, and reconstruction procedures.

Methods: Utilizing a bio-psycho-social approach of the International Classification of Functioning, Disability, and Health (ICF) model, this cross-sectional study (Ethical approval

reference number: 21/WA/0027; IRAS ID: 291087 – 10 years) incorporates questionnaires, individual ICF assessments, interviews, 3D Accelerometery and gait analysis.

The study recruited 91 participants over 19 months, (82 adults, 9 children; 59 males, 32 females; 88 diagnosis of malignant tumour, 3 benign; 83 Lower Limb, 8 Upper Limb Surgeries) with mean age 46.84± 21.12. These included osteosarcoma(n=29: 31.8%), chondrosarcoma(n=17: 18.6%), ewings sarcoma (n=6: 6.6%) and other diagnoses(n=39: 42.8%) and were recruited 65.14+-71.41 months post-surgery. A selected cohort underwent gait assessment in motor learning laboratory, activity monitoring with 3D accelerometer and qualitative interviews. Data were analysed using SPSS or thematic analysis.

Results: The mean scores of Toronto Extremity Limb Salvage Score(TESS) [670.39±21.43], Quality of Life for Cancer Survivors (QOL-CS) [5.69±1.59], EuroQol Questionnaire(EQ-5D) [66.93 ± 19.78 - Index: 21222] and modified Re-integration to Normal Living (mRNL) [70.09±24.53] suggested poorer outcomes than reference values. ICF assessment revealed patient specific participation restriction and barriers related to work environment, use of public transport, acceptance of limitations.

Data for community accelerometery (n=26) demonstrated variability in daily averages of step counts(mean=6117±3961 steps/day), energy expenditure 32.8± 1.6 metabolic equivalents (METs). Higher bouts of inactivity, lower step count was present in patients with secondary complications reflecting reduced activity levels.

Qualitative interviews (n=20) revealed the need for improved psychological support and post-surgery expectation information. Gait analysis performed on patients who underwent pelvic surgeries showed poorer posterior and medial balance and statistically significant difference in gait profile score when compared with healthy individuals.

Conclusion: The initial findings indicate that patients treated for musculoskeletal tumours experience moderate impacts on their quality of life and gait, with those experiencing secondary complications being more prone to inactivity and diverse impairments encompassing functional, environmental, and psychological aspects. This feasibility study also demonstrated STOMP as a versatile platform for comprehensive outcome collection in musculoskeletal tumour patients, allowing tailored assessments.



Fig.1: Data from Activity monitor of a 66 year old female patient who had an Above knee amputation for a musculoskeletal tumour in the right lower limb, demonstrating sedentary outcomes reflected by the higher sitting time (596.2 minutes) and a lower step count/day (1605).

(P 498) DEDIFFERENTIATED LIPOSARCOMA OF THE EXTREMITIES: CHARACTERISTICS AND OUTCOME

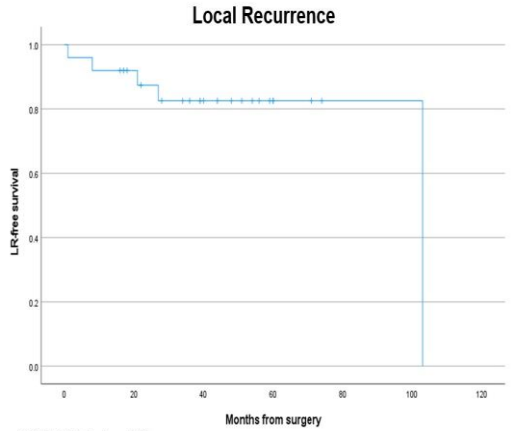
Jay Hoon Park, MD[1]; Han-soo Kim, N/A, MD. PhD.[2]; Shinn Kim, MD[1]; Yongsung Kim, Dr.[3]; Jeonghwan Youk, MD. PhD.[1]; Ilkyu Han, MD. PhD.[1], (1) Seoul National University Hospital, Seoul, Seoul-t'ukpyolsi, Republic of Korea, (2) Seoul National University Hospital, 101, Daehak-ro, Jongno-gu, Seoul-t'ukpyolsi, Republic of Korea, (3) Seoul National University Bundang Hospital, Gyunggi-do, Kyonggi-do, Republic of Korea

Objective: Literature on dedifferentiated liposarcoma of the extremities (DDL-E) is rare and its features have not been clearly elucidated. In this study, we aimed to investigate the characteristics and the outcome of DDL-E.

Methods: Twenty-five patients diagnosed with DDL-E from July 2004 to December 2020 were analyzed retrospectively. Demographic characteristics, histopathologic findings, fatty composition on magnetic resonance imaging (MRI), and treatment methods were collected. Oncologic outcomes (local recurrence-free survival (LRFS), metastasis-free survival (MFS) and disease-specific survival (DSS)) and prognostic factors were analyzed.

Results: There were 16 men and 9 women. The median age was 65 years (range, 35-87). The most common site of involvement was the thigh (n=22). The mean tumor size was 11.1 centimeters (cm) (range, 2.8-22.8). Eight (32%) presented after unplanned excision and 4 (12%) had metastases at presentation. Two had non-pulmonary organs (pancreas, peritoneum) as the first target of metastasis. Two patients (8%) had documented transition from well-differentiated liposarcoma (WDL) into DDL after 2 and 10 years of follow-up. Tumors were diagnosed as DDL-E when they were positive for MDM2 or CDK4, even without a WDL component. Based on the spectrum of fatty composition on MRI, 6 patients (24%) had well-defined fatty mass juxtaposed to well-defined non-fatty mass (type I), 2 (8%) had focal non-fatty component within a large fatty mass (type II), 9 (36%) had focal fatty component within a large non-fatty mass (type III), and 8 (32%) had non-fatty mass (type IV). As for FNCLCC grading, there were 2, 18, and 2 patients in grade 1, grade 2, and grade 3, respectively. Eleven patients underwent postoperative radiotherapy while two had postoperative chemotherapy. Histologically negative margin was achieved in 19 patients (73%).
Five patients (20%) had local recurrence and 5-year LRFS was 82.5%. In all, 7 patients (20%) had metastases. Of the 23 patients without initial metastases, 3 (12%) developed metastasis, and 5-year MFS was 92%. One patients (4%) died of disease at 2 months. Five year DSS was 96%. Local recurrence was associated with positive surgical margin (p=.012). DSS was associated with metastases (p=0.006). Fatty composition on MRI, tumors size or FNCLCC grade was not associated with oncologic outcome.

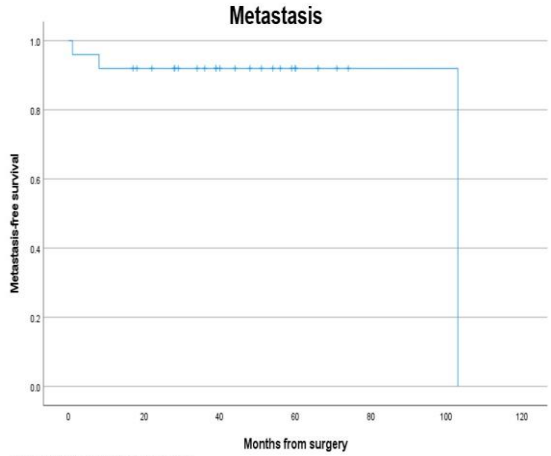
Conclusion: DDL-E without initial metastasis has a favorable oncologic outcome. Non-pulmonary organs are common first target of metastasis in DDL-E. Achieving negative surgical margin seems important for local control. Fatty composition on MRI, tumors size or FNCLCC grade was not associated with oncologic outcome.



20% (5 of 25) developed LR

Median Follow up was 45 months (2~124)

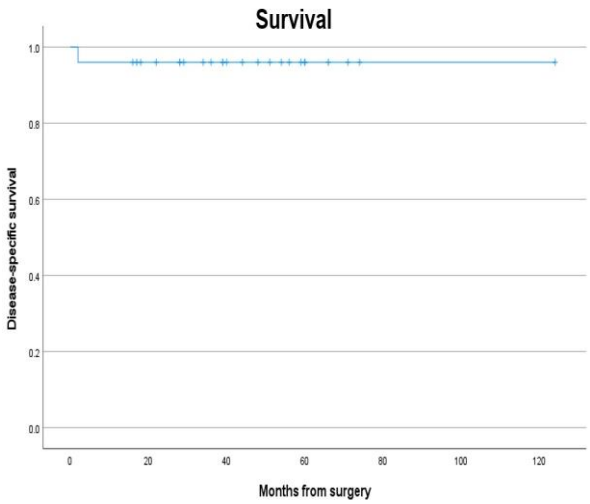
5-year LR-free survival was 82.5%



12% (3 of 25) developed Metastasis

Median Follow up was 45 months (2~124)

5-year Metastasis-free survival was 92.0%



4% (1 of 25) died of the disease

5-year, 10-year disease-specific survival was 92.0%

(P 499) CLINICAL OUTCOMES OF TOTAL FEMORAL REPLACEMENT FOR ONCOLOGICAL INDICATIONS

Jay Hoon Park, MD[1]; Shinn Kim, MD[1]; Yongsung Kim, Dr.[2]; Ilkyu Han, MD. PhD.[1]; Jeonghwan Youk, MD. PhD.[1]; Han-soo Kim, N/A, MD. PhD.[3], (1) Seoul National University Hospital, Seoul, Seoul-t'ukpyolsi, Republic of Korea, (2) Seoul National University Bundang Hospital, Gyunggi-do, Kyonggi-do, Republic of Korea, (3) Seoul National University Hospital, 101, Daehak-ro, Jongno-gu, Seoul-t'ukpyolsi, Republic of Korea

Objective: Total femoral replacement (TFR) is a salvage arthroplasty procedure used as an alternative to lower limb amputation. TFR can be performed for oncological and non-oncological indications. In the literature to date, only a few case series have described the results and complications of TFR for oncological indications. In this study, we aimed to investigate the clinical outcomes of TFR in oncological indications.

Methods: A total of 28 patients who underwent 31 total femoral replacements were identified from the database of our institute. All patients underwent primary or revision TFR for oncological reasons. Medical records and images were retrospectively reviewed.

Results: After a mean follow-up of 53.6 (3.2 to 157) months, 24 patients were still alive, whereas 4 patients died of malignant disease. Of the 31 cases, 3 were primary total femoral replacements and 28 were revisions of previous reconstructions. MUTARS, Stryker and LINK prostheses were used in 22, 5 and 1 cases respectively. A combination of MUTARS and Stryker prostheses was used in three cases. The reasons for primary TFR were tumor involvement of the whole femur (n=2) and skip metastasis of the tumor (n=1). The reasons for revision total femoral replacement were mechanical failure of previous salvage reconstruction (n=9), periprosthetic fracture with lack of bone stock (n=3), aseptic loosening (n=3), deep infection (n=7), tumor progression (n=4), aseptic loosening (n=1), correction of leg length discrepancy after total femoral replacement (n=1) and other. The mean Musculoskeletal Tumor Society score was 72.7% (range 30.0 to 90.0). There were a total of 8 (25.8%) complications requiring revision surgery: soft tissue failure (n=3), infection (n=3), failure of the hinge knee locking system (n=1), tumor progression (n=1). There were no cases of aseptic loosening. Revision-free implant survival at five years was 81.7% (95% CI 68.3% to 97.8%).

Conclusion: For oncological indications, TFR is a feasible surgical option in primary and revision limb salvage procedures. In terms of function and complications, the outcome of TFR is comparable to that of tumor prosthesis arthroplasty

(P 500) IMPROVING OUTCOMES OF INTERCALARY EWING SARCOMA RESECTIONS AND ALLOGRAFT RECONSTRUCTIONS WITH PATIENT-SPECIFIC CUTTING GUIDES AND VIRTUAL SURGICAL PLANNING

Thomas J. Utset-Ward, MD, MBA[1]; Thomas J. Scharschmidt, MD[2]; Daryn Strub, BA[2], (1) Nationwide Children's Hospital, Worthington, OH, (2) Nationwide Children's Hospital, Columbus, OH

Objective: We report our early experience with 3D-printed patient- and allograft-specific instrumentation for the resection and allograft reconstruction through a series of patients treated with intercalary resections of Ewing Sarcoma. With intercalary resection of bone sarcomas in pediatric patients, maintaining the integrity of the physes and not spanning the physis with the reconstruction are critical to the biologically growing limb salvage. Matching the allograft to the native bone at the junctions in selection and at the time of osteotomy aids in healing and eventual incorporation. We aim to evaluate improvements in union, margin status and reduction in revision reconstruction with the use of 3D printed cutting guides for the resection osteotomies and preparation of the allograft. We describe preoperative virtual planning and intraoperative techniques to best leverage this emerging technology and solve classic limitations with short fixation segments around the physis.

Methods: A case series of 21 patients who underwent intercalary resection and allograft reconstruction for Ewing sarcoma, including 13 patients treated with the use of custom 3D-printed cutting guides for the resection with matching cutting guides for the allograft, and 8 patients for which the guides were not used. Over a mean follow-up of 2.8 years, complications including non-union and revision reconstruction were collected in both groups.

Results: Reconstructions using this innovative instrumentation more likely to create better junction fit (OR 1.62, 95% CI 1.18, 2.06] and a lower rate of revision reconstruction (14% vs 75%). Bone marrow margins were negative in all cutting guide cases, but positive in 2 of the free-handed osteotomy resections. We describe techniques to maximize allograft fit and plan physeal-sparing fixation by simulating and pre-selecting hardware as well as pre-contouring implants.

Conclusion: Our early experience with 3D-printed cutting guides for intercalary resection and reconstruction in primary bone sarcomas demonstrates the potential to improve resection and bone-allograft fit. Osteotomies made in multiple planes with the 3D-printed cutting guides also provided more surface area for contact at the junctions. Using our method we have also been able to pre-select and pre-contour fixation implants preoperatively, reducing operative time and additional manipulation of the final allograft. There are additional opportunities to improve virtual surgical planning, implant selection and preparation for complex intercalary resections and reconstructions.

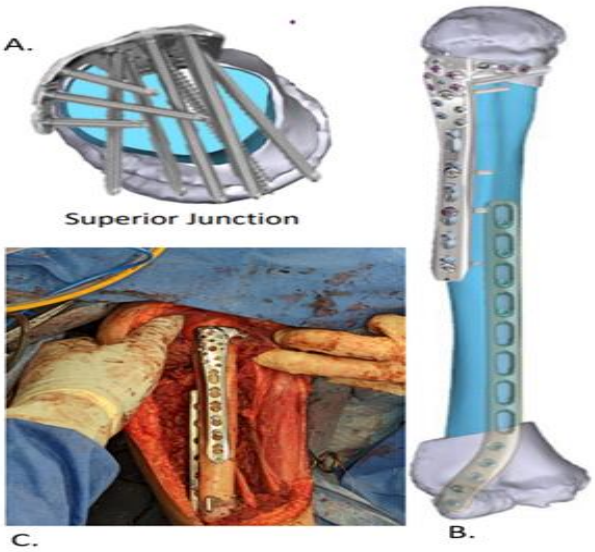


Fig 1. The process of patient and allograft specific cutting guide creation allows for additional virtual surgical planning including A. assessment of bone allograft junction B. testing atypical plates for C. physeal-sparing reconstructions

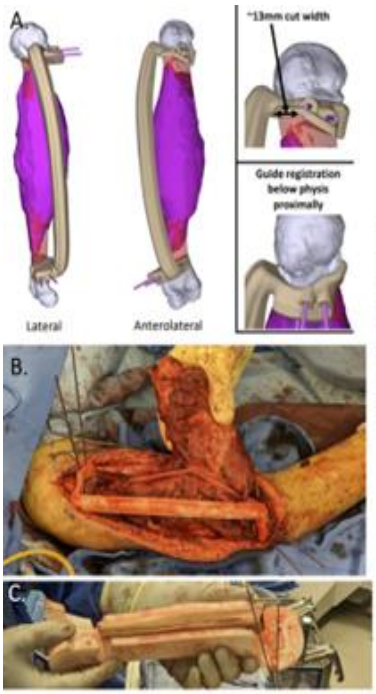


Fig 2. A. Virtual planning and design of the resection patient-specific cutting guide with additional planes and physeal-sparing cut of the proximal margin. B. Intraoperative photo following guide-assisted osteotomies and resection of the bone sarcoma C. Allograft specific cutting guide and cradle allowing proper preparation of the allograft.

(P 501) TIBIAL DEFORMITIES AND PHYSEAL INJURY WITH DISTAL FEMORAL EXPANDABLE ENDOPROSTHESIS IMPLANTS

Thomas J. Utset-Ward, MD, MBA[1]; Logan Good, BS[2]; Thomas J. Scharschmidt, MD[3]; Daryn Strub, BA[3], (1) Nationwide Children's Hospital, Worthington, OH, (2) The Ohio State University, Columbus, OH, (3) Nationwide Children's Hospital, Columbus, OH

Objective: In our use of the JTS expandable distal femoral replacement (eDFR), we have noticed acquired proximal tibial deformities. Studies have demonstrated disruptions in tibial growth following implantation, but none has investigated the exact physeal disruption. We aim to investigate if the location of the tibial stem entry in the physis, tibial tray alignment and cement placement will predict the development of the characteristic proximal tibia deformities.

Methods: A total of 13 patients receiving a JTS distal femoral replacement from 2014-2022 were included. In all cases the operative diagnosis was oncologic. We retrospectively reviewed preoperative and post-operative imaging to measure stem placement in the physis, alignment of the proximal tibia, and limb discrepancies. We used CTs and pre-operative image analysis with 3D modeling of the custom prostheses and mapped the tibial stem footprint to a novel gridded map of the proximal tibial physis.

Results: A proximal tibial deformity was noted in 6 of the 13 (46%) cases. The most common proximal tibial deformity was proximal tibia varus. However, in most cases deformity did not affect overall limb alignment. We are currently analyzing the exact location of physeal entry of the stem and the tray alignment at implantation.

Conclusion: With the current JTS eDFR implant design, the area of the proximal physis that is injured is determined by an implant stem position dictated by the intramedullary canal of the tibia. Further studies are needed to better understand the incidence of this deformity and contributing factors of implant design and patient anatomy.

(P 502) SEPARATE RESECTION OF BIOPSY TRACT IN PRIMARY BONE SARCOMA: IMPLICATIONS FOR LOCAL RECURRENCE AND OVERALL SURVIVAL

Thomas J. Utset-Ward, MD, MBA[1]; Dashaun Ragland, B.S.[2]; Thomas J. Scharschmidt, MD[3]; Michael D. Eckhoff, MD[4], (1) Nationwide Children's Hospital, Worthington, OH, (2) The Ohio State University, New York, NY, (3) Nationwide Children's Hospital, Columbus, OH, (4) Ohio State University, Columbus, OH

Objective: Including the biopsy tract with primary resections may limit operative options, potentially contributing to higher morbidity. This study aims to explore whether resecting biopsy tracts separately from the primary wide tumor resection specimen has any effect on local recurrence and overall survival.

Methods: This is a retrospective single-center study from 2014 to 2023. A total of 45 pediatric sarcoma cases were included with diagnoses of osteosarcoma (n=30), Ewing sarcoma (n=11), other primary bone sarcoma (n=4). Of these, the biopsy tract was resected separately from the main specimen in 24 (53%) cases.

Results: We noted a total local recurrence of 6.7% pediatric bone sarcomas. For cases with separate biopsy resection, 4.3% (n=1) demonstrated evidence of disease in the separate biopsy tract specimen. Separate resection of the biopsy tract did not affect the rate of local recurrence (OR 0.88, 95% CI 0.11, 6.77, p=0.90). There was no association between local recurrence and open vs core-needle biopsy (OR 0.28, 95% CI 0.35,2.29, p=0.24). Positive margins in the primary resected specimen (18% of cases) was an independent risk factor for the development of local recurrence.

Conclusion: There was no increased risk of local recurrence in cases where the biopsy tract was resected separately compared to cases where it was resected with the main tumor. Positive margins in the primary resection specimen was the greatest predictor of local recurrence, so optimizing surgical approach and resection strategy to best ensure negative margins is paramount.

Biopsy Information	Total (n=45)	Separate Biopsy Tract (n=24)	No Separate Biopsy Tract (n=21)
TYPE			
Open Biopsy	34 (75.6%)	21 (87.5%)	13 (61.9%)
Core Needle	11 (24.4%)	3 (12.5%)	8 (38.1%)
SPECIALTY PERFORMING BIOPSY			
Ortho	38 (84.4%)	23 (95.8%)	15 (71.4%)
Radiology	6 (13.3%)	1 (4.2%)	5 (23.8%)
Other	1 (2.2%)	0	1 (4.8%)
POSITIVE MARGINS	8 (17.8%)	3 (12.5%)	5 (23.8%)
LOCAL RECURRENCE	4 (8.9%)	2 (8.3%)	2 (9.5%)

Table 2

(P 503) SCAPULAR PROSTHESIS RECONSTRUCTION, EXPERIENCE IN OUR INSTITUTION

Laura Trullols Tarrago, MD[1]; Ana Peiro Ibañez, MD[2]; Pau Machado Granados, MD[2]; José Miguel Mata Muñoz, MD[2]; Manel Fa Binefa, MD[2]; Laura Garcia-Fontes Pujol, MD[2]; Sonia Casanova Zaragoza, Nurse[2]; Antonio López Pousa, MD[3]; Ana Sebio, MD, PhD[3]; Raul Teres, MD[2]; Silvia Bague, MD, PhD[2]; Ruth Orellana, MD[2]; Caterina Fumagalli, MD[2]; Jaume Llauger, Dr.[2]; Sandra Valverde, Dr.[2]; Oksana Lushchenkova, Dr.[2]; Claudia Nunez, MD PhD[2]; Diana Hernandez Jover, MD[2]; Jorge Enrique Otero Ruiz, MD.[4]; Katarina Majercakova, MD[2]; Antonio Vila Capel, MD[2]; Josep Isern Verdu, MD[2]; Manuel Fernández Garrido, MD/PhD[2]; Paul David

Zamora, Sr., MD[2]; Juan Carlos Trujillo-Reyes, MD/PhD[2]; Elisabeth Martinez-Tellez, MD/PhD[2]; José Antonio Gonzalez, MD, PhD[5]; Romina Ribas Carbonell, MD[2]; Mireia Solans Solerdelcoll, MD[2]; Isidro Gracia Alegria, MD, PhD[2], (1) Hospital de la Santa Creu i Sant Pau, Barcelona, Barcelona, Catalonia, Spain, (2) Hospital de la Santa Creu i Sant Pau, Barcelona, Catalonia, Spain, (3) Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain., Barcelona, Catalonia, Spain, (4) Hospital de la Santa Creu i Sant Pau, Sant Adria de Besos, Catalonia, Spain, (5) Department of Surgery, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, Barcelona, Catalonia, Spain

Objective: Scapular tumors are very rare. Incidence is unclear due to a number of factors, including undiagnosed and unreported tumors. More than 50% of scapular bone tumors will correspond to malignant entities, most of them chondrosarcoma or metastases.
Currently, 95% of patients with sarcomas in the shoulder region are treated with surgical techniques that allow a limb salvage surgery together with a safe tumor resection, for example the Tikhoff-Linberg procedure and their modifications.
But in addition to the correct tumor excision, it is necessary to take into account restoring the stability of the shoulder in order to preserve correct elbow and hand functionality along with acceptable aesthetics.
We present our experience in total scapulectomy resections reconstructed with a scapular prosthesis implant.

Methods: This is a descriptive study, analyzing the oncological and functional results of patients affected by scapular sarcomas who required surgical treatment consisting in total scapulectomy and reconstruction with scapular prosthesis, in our institution. Minimum follow-up required was 12months. We applied the Constant Score (especific standardized shoulder test) at 3-6-12-18-24 months postoperatively.

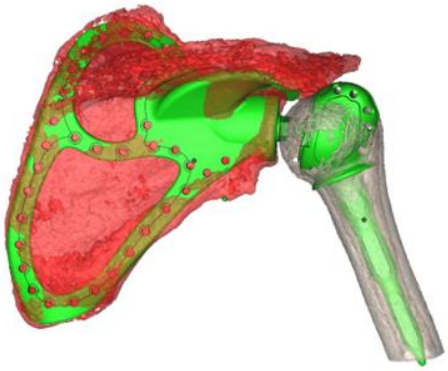
Results: There were 6 patients (2 men, 4 women), mean age of 31 years (17-46), mean follow-up 39 months (18-56). Diagnoses: 2/6 Ewing sarcoma, 1/6 osteosarcoma, 1/6 chondrosarcoma, 1/6 leiomyosarcoma, 1/6 alveolar sarcoma. All of them reconstructed by total scapular prosthesis with retentive proximal humerus implant. No postoperative complications, no infections, no luxation. In 4/6 the scapula prosthesis was placed in primary surgery procedure, while in the other 2/6 patients, was implanted after resection surgery of tumor recurrence.

Oncological results: resection piece showed less than 15% of tumoral viability in 2/6 patients. These patients are alive and disease free at the end of the study.

Functional results: we obtained results ranging from 51-83% in the Shoulder Constant Score. We observed that the function improves progressive and constantly throughout the follow-up.

Conclusion: The scapula bone has several stabilizing ligaments and 17 muscle attachments, which is why it contributes significantly to the mobility of the shoulder, the joint with most ranges of motion in the body.
In addition, the scapula is part of the shoulder girdle, which has the function of anchoring the upper extremity to the axial skeleton.
The fact of reconstructing the scapula with a prosthetic implant not only allows us to maintain anatomical musculoskeletal continuity, but also restores the offset of the humerus. This enables a correct position of the elbow and hand, and an acceptable-good functionality, as it is reflected in the results obtained in the Shoulder Constant Score.
The use of retentive / constrained implants and reconstruction meshes restoring gleno-humeral

joint, increases static stability and reduces the rate of dislocations.
According to our experience, the reconstruction system using total scapular prosthesis after a total scapulectomy procedure, offers several advantages compared to non-reconstruction:
1.- increase the range of glenohumeral mobility
2.- increase strength and load capacity in the ipsilateral elbow and hand
3.- prevents floating shoulder and therefore the symptoms derived from brachial plexus traction
4.- aesthetic result of the shoulder relief







(P 504) WHY DO SOME PATIENTS WITH SYNOVIAL SARCOMA HAVE BETTER FUNCTIONAL OUTCOMES AFTER RESECTION SURGERY? ANALYSIS OF 102 CASES

Ana Peiro Ibañez, MD[1]; Laura Trullols Tarrago, MD[2]; Ana Sebio, MD, PhD[3]; Elisabeth Martinez-Tellez, MD/PhD[1]; Katarina Majercakova, MD[1]; Manuel Fernández Garrido, MD/PhD[1]; Silvia Bague, MD, PhD[1]; José Antonio Gonzalez, MD, PhD[4]; Ruth Orellana, MD[1]; Caterina Fumagalli, MD[1]; Juan Carlos Trujillo-Reyes, MD/PhD[1]; Paul David Zamora, Sr., MD[1]; Raul Teres, MD[1]; Antonio López Pousa, MD[3]; Claudia Nunez, MD PhD[1]; Jorge Enrique Otero Ruiz, MD.[5]; Sandra Valverde, Dr.[1]; Oksana Lushchenkova, Dr.[1]; Antonio Vila Capel, MD[1]; Josep Isern Verdu, MD[1]; MariaJesus quintana, Dr.[1]; Diana Hernandez Jover, MD[1]; Jaume Llauger, Dr.[1]; Isidro Gracia Alegria, MD, PhD[1], (1) Hospital de la Santa Creu i Sant Pau, Barcelona, Catalonia, Spain, (2) Hospital de la Santa Creu i Sant Pau, Barcelona, Barcelona, Catalonia, Spain, (3) Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain., Barcelona, Catalonia, Spain, (4) Department of Surgery, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, Barcelona, Catalonia, Spain, (5) Hospital de la Santa Creu i Sant Pau, Sant Adria de Besos, Catalonia, Spain

Objective: Synovial sarcoma (SS) is a very aggressive mesenchymal tumor with partial epitelial differentiation and a high potential for metastasis. The presence of t(X;18)(p11.2;q11.2) is a pathognomonic feature of synovial sarcoma. SS is considered the fourth most common sarcoma at any age. It's prognosis is very por with an expected 5-year survival between 50-60% in adults. Survivors must face with major functional sequelae secondary to large muscle resections and adjuvant treatments such as radiotherapy. Functional outcomes are important for oncologic patients undergoing limb sarcoma surgery. The aim of this study is to identify predictors of postoperative function in musculoskeletal oncology patients that came through a synovial sarcoma resection surgery.

Methods: We retrospectively reviewed 102 consecutive cases of synovial sarcoma treated in our institution between 1983 - 2016. We went over multiple variables related to the most commonly used functional outcome scales (MSTS-ISOLS score) in order to analyze possible risk factors for poor functional outcomes. For hypothesis testing, after confirming the normality of the distributions in the numerical variables using the Kolmogorov-Smirnoff test, we used ANOVA and the parametric Student T-test. For qualitative variables, we used Pearson's Chi-square test or Fisher's exact test.

Results: 102 cases were included in our study. 60 men (58.8%) and 42 women (41.2%). The mean age at diagnosis was 37.9 years ±17.5 (10-80). In our sample the third decade of life was the one with the highest incidence of synovial sarcoma cases followed by the fifth decade of life. Regarding to the functional outcomes, no statistically significant differences were observed with respecto to gender, location, histological variant or degree of differentiation, the time elapsed between symptom onset and first consultation, diagnosis or initiation of treatment. Statistically significant differences were observed according to the mean age of the patients with improvement in the youngest ($p=0.036$), specially in patients under 25 years. Statistically significant differences were also observed for the functional scale results ($p< 0.001$) according to the AJCC staging. With regard to size, both the largest tumor diameter (measured in centimeters) ($p=0.014$) and tumor volume (measured in cubic centimeters) ($p=0.03$) were statistically significant regarding functional outcomes. Statistically significant differences were observed in cases with no local recurrence ($p< 0.001$) and in those with no distant metastases ($p< 0.001$). Comparatively and also statistically significantly ($p< 0.001$), the surgical technique

implies the functional results, worse functional results are to be expected in amputations compared to limb salvage. Statistically significant differences were observed with respect to treatment (TABLES 226-227) with chemotherapy ($p< 0.001$) and with respect to combined treatment by surgery, radiotherapy (RDT) and chemotherapy (QT) ($p< 0.001$) in favor of the association to chemotherapy treatment. However, no significant differences were obtained with respect to functional outcomes and the association of radiotherapy with surgical treatment.

Conclusion: Younger patients, patients undergoing limb salvage compared to amputation, patients with smaller tumor, those treated with chemotherapy or combined treatment, and patients without recurrences or dissemination, presented better functional results after undergoing synovial sarcoma surgery.

(P 505) LOCAL RECURRENCE IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS: A MULTICENTER COHORT STUDY

Christianne Jansma, Bsc[1]; Ibtissam Acem, Bsc[2]; J. Henk Coert, MD, PhD[3]; Walter Taal, MD, PhD[2]; Dirk J. Grünhagen, MD, PhD[2]; Cornelis Verhoef, MD, PhD[2]; Enrico Martin, MD, PhD[3], (1) Erasmus Medical Center and UMC Utrecht, Amsterdam, Noord-Holland, Netherlands, (2) Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands, (3) UMC Utrecht, Utrecht, Utrecht, Netherlands

Objective: Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are rare and aggressive malignant soft-tissue sarcomas (STS) and compromise 5-10% of STS. About 40% of MPNST cases are associated with neurofibromatosis type 1 (NF1). High-grade MPNSTs have a high probability rate of a local recurrence (LR), and overall, MPNSTs are the most recurrent tumors within all types of STS. The development of a LR is associated with a morbid event that decreases functional outcomes in MPNST patients. Risk factors vary in current literature due to its rarity, as does the optimal treatment for LRs and its impact on overall survival (OS).

Methods: Surgically treated primary MPNSTs between 1988 to 2019 in the MONACO multicenter cohort were included. Multivariate Cox regression analyses were performed to identify factors associated for development of LR and OS after LR. Treatment of LR was evaluated.

Results: A total of 507 patients were included, of which 142 developed a first LR (28%). High-grade (HR 2.63; 95% CI, 1.15-5.99), microscopically positive margins (R1) (HR 2.19; 95% CI, 1.51-3.16), and large tumor size (HR 2.14; 95% CI, 1.21-3.78) were independent risk factors for the development of a LR. Radiotherapy (HR 0.62; 95% CI, 0.43-0.89) on the other hand, reduced the risk. Patients with a LR had significantly worse OS. In patients with a LR, synchronous metastasis was associated with poor OS (HR 1.79; 95% CI 1.02-3.14) while surgically treated LRs had a better OS (HR 0.38; 95% CI 0.22-0.64). Most LRs (64.9%) were treated surgically without additional therapy. Radiotherapy combined with surgery was administered in 11.3% of patients with a LR.

Conclusion: Large, high-grade MPNSTs with R1 resections are at increased risk of developing a LR, while the use of radiotherapy decreases this risk. Surgical treatment of LR is associated with improved OS.

Table. Univariate and multivariate analysis of risk factors for the development of a first local recurrence				
	Univariate		Multivariate	
Variables	HR (95% CI)	p Value	HR (95% CI)	p Value
Age (per 10 years)	1.01 (0.928-1.11)	0.767		
NF1				
No	1.00	-	1.00	-
Yes	1.49 (1.04-2.13)	0.030	1.14 (0.779-1.66)	0.507
Tumor grade				
Low grade	1.00	-	1.00	-
High grade	2.38 (1.10-5.16)	0.032	2.63 (1.15-5.99)	0.026
Tumor size				
<5 cm	1.00	-	1.00	-
>5 cm	2.45 (1.47-4.08)	0.001	2.14 (1.21-3.78)	0.011
Triton				
No	1.00	-		
Yes	0.683 (0.271-1.73)	0.424		
Tumor depth				
Superficial	1.00	-	1.00	-
Deep	1.41 (0.841-2.37)	0.198	1.07 (0.607-1.90)	0.807
Site of primary tumor				
Head and neck	1.00	-	1.00	-
Extremities	1.26 (0.717-2.21)	0.425	1.10 (0.593-2.03)	0.768
Central	1.65 (0.934-2.93)	0.087	1.28 (0.682-2.38)	0.447
Margin primary tumor				
R0	1.00	-	1.00	-
R1	2.06 (1.45-2.93)	<0.001	2.19 (1.51-3.16)	<0.001
Radiotherapy primary tumor				
No	1.00	-	1.00	-
Yes	0.809 (0.544-1.14)	0.230	0.616 (0.426-0.892)	0.012
Chemotherapy primary tumor				
No	1.00	-		
Yes	0.897 (0.544-1.48)	0.669		

NF1: neurofibromatosis type 1, cm: centimeter

Table. Treatment of recurrence	
Variable	**LR1**
N	142
Time to local recurrence	
Mean (SD)	23.29 (34.98)
Surgery for LR1/LR2	
No	44 (31.0%)
Yes	92 (64.8%)
Unknown	6 (4.2%)
Surgical margin	
R0	37 (37.8%)
R1	13 (13.3%)
R2	3 (3.1%)
Unknown	45 (45.9%)
Treatment of LR1/LR2	
No treatment	29 (20.4%)
Surgery	72 (50.7%)
Surgery + RTX	16 (11.3%)
Surgery + CTX	3 (2.1%)
Surgery + RTX + CTX	1 (0.7%)
RTX	8 (5.6%)
CTX	7 (4.9%)
Unknown	6 (4.2%)
Radiotherapy	
No	65 (45.8%)
nRTX	4 (2.8%)
aRTX	21 (14.8%)
Unknown	52 (36.6%)
Chemotherapy	
No	80 (56.3%)
nCTX	3 (2.1%)
aCTX	7 (4.9%)
Both	1 (0.7%)
Unknown	51 (35.9%)

N: number of patients, RTX: radiotherapy, CTX: chemotherapy, LR1: first local recurrence, nRTX: neoadjuvant radiotherapy, aRTX: adjuvant radiotherapy, nCTX: neoadjuvant chemotherapy, aCTX: adjuvant chemotherapy

Table. Univariate and multivariate analysis of risk factors for overall survival in patients with a first local recurrence

	Univariate		Multivariate	
Variables	HR (95% CI)	p Value	HR (95% CI)	p Value
Age (per 10 years)	1.05 (0.938-1.17)	0.415		
NF1				
No	1.00	-		
Yes	0.98 (0.614-1.53)	0.938		
Tumor grade				
Low grade	1.00	-	1.00	-
High grade	2.35 (1.42-3.88)	0.001	2.06 (0.846-5.00)	0.121
Tumor size				
<5 cm	1.00	-	1.00	-
>5 cm	1.66 (0.815-3.38)	0.170	1.24 (0.59-2.61)	0.573
Tumor depth				
Superficial	1.00	-	1.00	-
Deep	2.30 (1.42-3.71)	0.001	1.98 (0.985-3.96)	0.061
Site of primary tumor				
Head and neck	1.00	-		
Extremities	1.13 (0.570-2.25)	0.723		
Central	1.37 (0.681-2.74)	0.382		
Margin primary tumor				
R0	1.00	-	1.00	-
R1	1.35 (0.880-2.07)	0.174	1.08 (0.654-1.78)	0.769
Radiotherapy primary tumor				
No	1.00	-		
Yes	1.27 (0.830-1.94)	0.275		
Surgery LR1				
No	1.00	-	1.00	-
Yes	0.364 (0.238-0.557)	<0.001	0.375 (0.221-0.636)	<0.001
Margin LR1				
R0	1.00	-		
R1	1.39 (0.743-2.62)	0.307		
R2	0.777 (0.425-1.42)	0.418		
Radiotherapy LR1				
No	1.00	-	1.00	-
nRTx	0.465 (0.275-0.784)	0.005	1.34 (0.526-3.40)	0.545
aRTX	0.583 (0.325-1.05)	0.075	0.752 (0.388-1.46)	0.404
Metastasis during LR1				
No	1.00	-	1.00	-
Yes	2.532 (1.62-3.97)	<0.001	1.79 (1.02-3.14)	0.046

NF1: neurofibromatosis type 1, cm: centimeter, LR1: first local recurrence, nRTX: neoadjuvant radiotherapy, aRTX: adjuvant radiotherapy

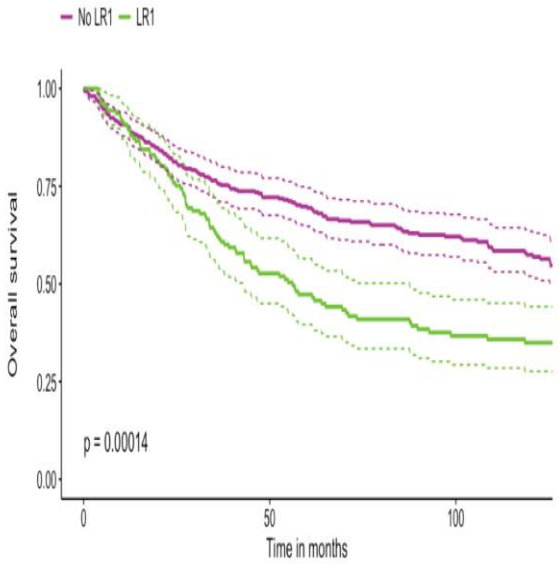


Figure 1. Survival plot of patients with and without a first Local Recurrence (LR1). *p*-value: Computed with log-rank test.

(P 506) THE EFFECT OF TIMING BETWEEN PRE-OPERATIVE EMBOLIZATION AND SURGERY: A RETROSPECTIVE ANALYSIS OF HYPERVASCULAR BONE METASTASES

Alexander J. Acuña, MD[1]; Gayathri Vijayakumar, BS[2]; Neil Buac, BS[2]; Ira Miller, MD, PhD[2]; Matthew Colman, MD[2]; Steven Gitelis, MD[2]; Alan T. Blank, MD, MS[2], (1) Rush University Medical Center - - Chicago, IL, Chicago, IL, (2) Rush University Medical Center, Chicago, IL

Objective: Pre-operative embolization for metastatic bone lesions has the potential to reduce intra-operative blood loss and improve perioperative outcomes in patients with hypervascular tumors. The optimal timing between embolization and surgery has yet to be established. Our analysis sought to evaluate how the timing of embolization (< 24 hours vs. >24 hours) prior to surgery impacts blood loss, transfusion risk, and operative time in patients with hypervascular primary tumors.

Methods: We identified patients with renal cell (RCC) or thyroid carcinoma undergoing surgery between 1992 and 2023 (Table 1). These patients were segregated into the following cohorts: 1) no embolization pre-operatively, 2) surgery within 24 hours of embolization, and 3) surgery greater than 24 hours after embolization. Our primary outcomes were estimated blood loss (EBL), operative time, perioperative transfusion rate, and change in hemoglobin during the perioperative period. Multivariate logistic regression analyses were performed to assess the effect of pre-operative embolization and its timing while controlling for patient age, American Society of Anesthesiology (ASA) Class, lesion size, type of procedure, and the presence of a pathologic fracture. For EBL and operative time, patients were segregated into high or low categories based on median values. For perioperative hemoglobin changes, patients were similarly segregated based on mean values.

Results: No differences were seen in all evaluated outcomes between immediate and delayed embolization cohorts (Table 2). When comparing those with and without embolization, no differences were seen in EBL, transfusion rates, or hemoglobin changes (Table 3). Patients without embolization had a significantly lower operative time. No differences in EBL were seen between the immediate (OR: 0.685, 95% CI: 0.159-2.949; p=0.611) and delayed (OR: 0.568, 95% CI: 0.093-3.462; p=0.539) surgery cohorts compared to patients without embolization. Surgery greater than 24 hours after embolization was not associated with a higher risk of prolonged operative time (OR: 13.499, 95% CI: 0.832-219.146; p=0.067). No differences in transfusion incidence or hemoglobin drop were seen between embolization cohorts.

Conclusion: Surgery within 24 hours of embolization did not significantly reduce blood loss, transfusion rate, operative time, or hemoglobin change compared to surgery at later time interval. Similarly, no differences in these outcomes were seen between patients with and without embolization. These findings suggest that surgery may be safely delayed beyond 24 hours from embolization without a higher risk of bleeding. Similarly, in appropriately selected cohorts, embolization may not be needed pre-operatively. No larger study has evaluated embolization timing among patients with metastases in various anatomic regions. It remains important to consider how selection bias may influence choices regarding embolization and procedures performed.

Table 1. Patient Demographics

Age	62 (IQR 59-68)
Sex	
Male	54 (70.1%)
Female	23 (29.9%)
Metastatic Cancer	
Thyroid Cancer	8 (10.3%)
Renal Cell Cancer	69 (89.6%)
Pre-operative Embolization	46 (55.4%)
Anatomic Location*	
Upper Extremity	25
Lower Extremity	51
Spine	7
Procedure Type	
Resection/Intralesional Excision	
No arthroplasty or internal fixation	12 (14.6%)
With Arthroplasty	29 (35.4%)
With Internal Fixation	18 (22.0%)
No Resection/Intralesional Excision	
Arthroplasty	7 (8.5%)
Internal Fixation	16 (19.5%)

*Three patients had multiple lesions

Table 2. Comparison Between Patients Based on Embolization Timing.

	Preop Embolization <24 hours	**Preop Embolization >24 hours**	***p*-value**
Total Number	32	14	
Tourniquet Used	0	1	p=0.289
Pathologic fracture	22	5	p=0.060
Location of Lesion	Upper extremity: 9 Lower extremity: 17 Spine: 6	Upper extremity: 5 Lower extremity: 9 Spine: 0	p=0. 360
Lesion Size (median, cm)	5 cm (IQR 2.55 -7.43)	5.2 cm (IQR 3.2 – 6.2)	p=0.948
Procedure Type*	Resection: 5 Resection and arthroplasty: 12 Resection and Internal fixation: 10 Arthroplasty: 4 Internal fixation: 1	Resection: 1 Resection and arthroplasty: 7 Resection and Internal fixation: 2 Arthroplasty: 1 Internal fixation: 2	p=0.417
Operative Time (median, min)	135 min (IQR 113.5-164.3)	155 min (IQR 130-183)	0.244
EBL (median, mL)	300 (IQR 200-500)	300 (IQR 250-400)	0.744
Difference between Preop and Postop Hemoglobin (mean)	1.62 +/-1.72	1.52 +/-1.24	p=0.846
Transfusion Intraop	5	4	p=0.411
Transfusion Postop	5	1	p=0.656
Return to OR during hospital stay	2	0	p=1.00
Readmission in <90 days	13	5	p=0.893

*, One patient had internal fixation of the upper extremity and lower extremity in the same procedure

Table 3. Comparison of Pre-Operative Embolization vs No Pre-Operative Embolization.

	Preop Embolization	No Preop Embolization	*p*-value
Total Lesions	46	37	
Tourniquet Used	1	7	**p=0.020**
Pathologic fracture	27	18	p=0.304
Location of Lesion	Upper extremity: 14 Lower extremity: 26 Spine: 6	Upper extremity: 11 Lower extremity: 25 Spine: 1	p=0.255
Lesion Size (median, cm)	5 cm (IQR 2.75 cm-6.95)	3.5 cm (IQR 2.5 cm-5.58)	p=0.197
Procedure Type*	Resection: 6 Resection and arthroplasty: 19 Resection and Internal fixation: 12 Arthroplasty: 5 Internal fixation: 3	Resection: 6 Resection and arthroplasty: 10 Resection and Internal fixation: 6 Arthroplasty: 2 Internal fixation: 13	**p=0.019**
Operative Time (median, min)	144 (IQR 115-173)	99.5 (IQR 75.8-129.8)	**p<0.001**
EBL (median, mL)	300 (IQR 200-500)	200 (IQR 100-500)	p=0.115
Difference between Preop and Postop Hemoglobin (mean)	1.59 +/-1.59	1.76+/-1.16	p=0.620
Transfusion Intraop	9	5	p=0.437
Transfusion Postop	6	3	p=0.503
Return to OR during hospital stay	2	0	p=0.499
Readmission in <90 days	18	11	p=0.333

*, One patient had internal fixation of the upper extremity and lower extremity in the same procedure

(P 507) PRIMARY CHEST WALL TUMORS OF MESENCHYMAL ORIGIN. EXPERIENCE OF A REFERENCE UNIT

Juan Carlos Trujillo-Reyes, MD/PhD[1]; Georgina Planas Canovas, Thoracic Surgeon[1]; Josep Belda Sanchis, MD,PhD[1]; Alejandra Libreros Niño, MD[1]; Sonia Stephania Chavarria Murillo, MD[1]; Manuel Fernández Garrido, MD/PhD[1]; Paul David Zamora, Sr., MD[1]; Silvia Bagué, MD[1]; Ruth Orellana, MD[1]; Laura Lopez, MD,PhD[2]; Caterina Fumagalli, MD[1]; Ana Sebio, MD, PhD[3]; Raul Teres, MD[1]; Antonio López Pousa, MD[3]; Claudia Nunez, MD PhD[1]; Diana Hernandez Jover, MD[1]; Jaume Llauger, Dr.[1]; Ana Peiro Ibañez, MD[1]; Pau Machado Granados, MD[1]; Isidro Gracia Alegria, MD, PhD[1]; Laura Trullols, MD[1]; José Antonio Gonzalez, MD, PhD[4]; Antonio Vila Capel, MD[1]; Josep Isern Verdu, MD[1]; Katarina Majercakova, MD[1]; Oksana Lushchenkova, Dr.[1]; Jorge Enrique Otero Ruiz, MD.[5]; Sandra Valverde, Dr.[1]; Elisabeth Martinez-Tellez, MD/PhD[1], (1) Hospital de la Santa Creu i Sant Pau, Barcelona, Catalonia, Spain, (2) Hospital de la Santa Creu i San Pau, Barcelona, Catalonia, Spain, (3) Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain., Barcelona, Catalonia, Spain, (4) Department of Surgery, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, Barcelona, Catalonia, Spain, (5) Hospital de la Santa Creu i Sant Pau, Sant Adria de Besos, Catalonia, Spain

Objective: Primary chest wall tumors of mesenchymal origin are rare representing only 1% of all malignant tumors in adulthood. Histologically they are a very heterogeneous group which makes the diagnostic process difficult.
The aim of this study is to analyze the data of patients diagnosed with primary tumor of mesenchymal origin who underwent surgery in a reference unit. In addition, we want to see

how histological type and type of resection influence overall survival and disease-free survival at 1, 3 and 5 years.

Methods: Observational and retrospective study to analyze the series of patients who underwent surgery with a diagnosis of primary chest wall tumor of mesenchymal origin during the period 2012-2022 (n=40 patients). Demographic variables, comorbidities, tumor size, histology and histological grade, type of resection, surgical margins and reconstruction technique, postoperative complications, adjuvant and/or neoadjuvant therapy, duration of follow-up and survival were recorded. An analysis of overall survival and disease-free survival at 1, 3 and 5 years was performed by log-rank test.

Results: The median age was 57 years (SD±19 years). Forty percent (N=16) had a previous oncologic history and 69% of patients had less than 2 comorbidities at the time of surgery. In 37 patients (93%) of the tumors operated on, the histological diagnosis was sarcoma, 13 patients (33%) were of bone origin and 27 patients (68%) were soft tissue sarcomas (Image 1). One patient was excluded from the study due to loss to follow-up.The median tumor size was 9 cm (SD±5.6cm). Fifty-three percent of the tumors (N=21) were of intermediate-high histologic grade. Resection was marginal in 40% of cases. Twenty-two patients required chest wall reconstruction. In 19 patients a synthetic or biological mesh was used and in 10 of them a rigid reconstruction with titanium rods (N=8) or titanium mesh (N=2) was also performed. In 17 patients a flap was used to cover the defect, the most commonly used being the pedicled latissimus dorsi flap (N=7). Flap infection resulted in bar removal in 2 patients. Twenty-five percent (N=10) underwent induction treatment and 45% (N=18) adjuvant treatment with chemotherapy and/or radiotherapy. Thirty percent (N=12) of the patients had local recurrence and 45% (N=18) had distant metastases. The median overall survival time was 68.1 months (95% CI 49.8-86.4). Overall survival at 1 year was 87%, at 3 years was 58% and at 5 years was 46%. The disease-free time was 15.9 months (CI 95% 3.3- 28.6) with 60% at 1 year, 40% at 3 years and 36% at 5 years (Table 1).
As influential factors in OS and DFS, the resection margin and histological type were analyzed. In spite of not obtaining a statistically significant difference, a tendency to lower overall survival was observed in tumors of soft tissue origin and in those where resection was not complete (Image 2).The same is true for disease-free survival. Tumors of soft tissue origin or those with marginal or incomplete resection had a shorter disease-free time (Image 3).

Conclusion: - In this group of patients, overall survival at 5 years was 46%, while disease-free survival at 5 years was 36%.
- Sarcomas with soft tissue origin and marginal or incomplete resection are two factors that negatively influence overall survival and disease-free survival.
- Given the low prevalence of these tumors, a multidisciplinary approach in reference units is essential to guarantee a correct diagnostic and therapeutic process.

Table 1

	Disease Free Survival (DFS) CI 95%	Overall Survival (OS) CI 95%
1 year after resection	60%	87%
3 years after resection	40%	58%
5 years after resection	36%	46%

OS and DFS 1,3 and 5 years after resection

Image 1

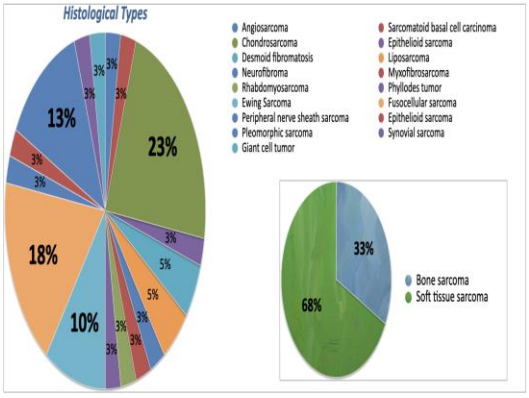
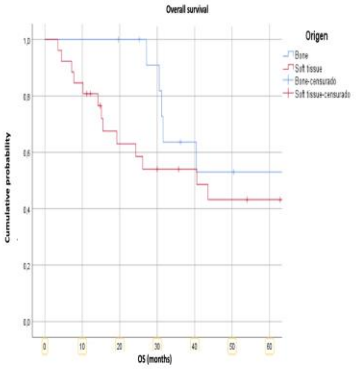


Image 2

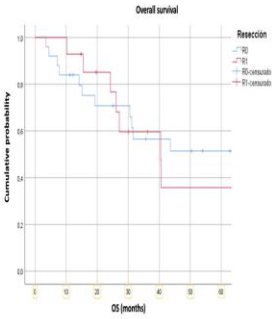
Overall Survival according to histology



	Total	Dead	Alive	Median (months)
Bone	13	5	8	83,9
Soft tissue	26	14	12	54,6

Log Rank (mantel-cox) p=0,212

OS according to complete or incomplete resection

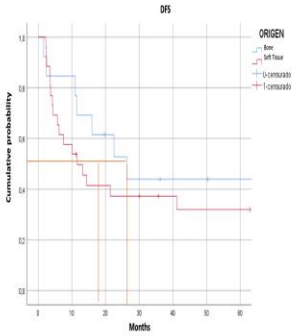


	Total	Dead	Alive	Median (months)
R0 (blue)	25	12	13	68,5
R1 (red)	14	7	7	54,9

Log Rank (mantel-cox) p=0,863

Image 3

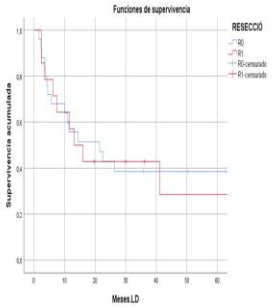
DFS according to histology



	Total	Recurrence	No recurrence	Median (months)
Bone	13	7	6	26,3
Soft tissue	26	17	9	11,6

Log Rank (mantel-cox) p=0,356

DFS according to complete or incomplete resection



	Total	Recurrence	No recurrence	Median (months)
R0	25	15	10	21,4
R1	14	9	5	13,2

Log Rank (mantel-cox) p=0,844

(P 508) DREAM, UTOPIA OR REALISM? PROGNOSTIC FACTORS IN LUNG METASTASECTOMY OF DISSEMINATED SARCOMA DISEASE

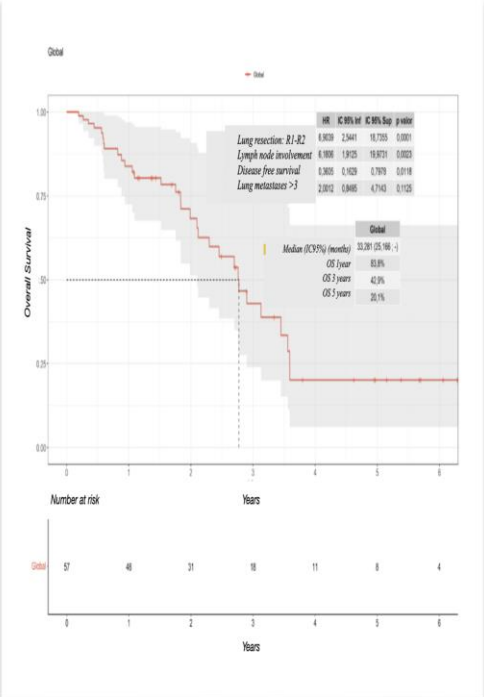
Elisabeth Martínez-Téllez, MD,PhD[1]; Juan Carlos Trujillo-Reyes, MD/PhD[1]; Georgina Planas Canovas, Thoracic Surgeon[1]; Alejandra Libreros Niño, MD[1]; Sonia Stephania Chavarria Murillo, MD[1]; Raul Teres, MD[1]; Ana Sebio, MD, PhD[2]; Silvia Bagué, MD[1]; Ruth Orellana, MD[1]; Vicenç Artigas Raventós, MD,PhD[3]; Antonio López Pousa, MD[2]; Laura Lopez, MD,PhD[4]; Katarina Majercakova, MD[1]; Paul David Zamora, Sr., MD[1]; Manuel Fernández Garrido, MD/PhD[1]; José Antonio Gonzalez, MD, PhD[5]; Ana Peiro Ibañez, MD[1]; Laura Trullols Tarrago, MD[6]; Isidro Gracia Alegria, MD, PhD[1]; Pau Machado Granados, MD[1]; Jaume Llauger, Dr.[1]; Sandra Valverde, Dr.[1]; Caterina Fumagalli, MD[1]; Antonio Vila Capel, MD[1]; Sonia Casanova Zaragoza, Nurse[1]; Diana Hernandez Jover, MD[1]; Josep Isern Verdu, MD[1]; Josep Belda Sanchis, MD,PhD[1], (1) Hospital de la Santa Creu i Sant Pau, Barcelona, Catalonia, Spain, (2) Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain., Barcelona, Catalonia, Spain, (3) Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain, (4) Hospital de la Santa Creu i San Pau, Barcelona, Catalonia, Spain, (5) Department of Surgery, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, Barcelona, Catalonia, Spain, (6) Hospital de la Santa Creu i Sant Pau, Barcelona, Barcelona, Catalonia, Spain

Objective: Patients with a primary tumor of sarcomatous origin may present distant metastases in up to 50% of cases, being the lung the main target organ. The selection of the subset of patients who underwent surgical resection is crucial. There are well-established criteria for pulmonary metastasectomy, but multiple factors will influence the patient's outcome and, also, these factors will play as prognostic factors for recurrence and survival. To identify which prognostic factors are associated with better survival after pulmonary metastasectomy.

Methods: The design of the study was observational, descriptive, and prospective of patients affected by lung metastases from sarcoma and who underwent pulmonary metastasectomy from June 2014 to January 2021. We analyzed different subgroups according to the histological subtype of sarcoma, surgical approach, type of lung resection, disease synchronicity, lymph node involvement, and disease free time.

Results: 57 patients were included. 34 men and 23 women with a mean age of 52.8 +/- 14 years. A total of 80 pulmonary metastasectomy were performed. In the multivariate analysis, we identified prognostic factors that improve overall survival (OS): performing a complete resection (R0), disease-free time longer than one year, the number of metastases resected less than 3, and the non-existence of lymph node involvement. The median overall survival was 33.2 months (95% CI 25.1 ; -). Overall survival one year after surgery was 84%, 43% at 3 years, and 20% at 5 years, respectively.

Conclusion: In our series, we have identified several prognostic factors that influence the OS of patients with lung metastases from sarcoma: performing a complete resection, disease-free time longer than twelve months before lung resection, the number of metastases resected less than 3 and the non-existence of lymph node involvement.



(P 509) GIANT CELL TUMOR OF BONE IN THE PEDIATRIC POPULATION: A RETROSPECTIVE STUDY HIGHLIGHTING CASES OF METAPHYSEAL ONLY LOCATION AND INCREASED LOCAL RECURRENCE RATES IN SKELETALLY IMMATURE PATIENTS

Troy Q. Tabarestani, BA[1]; Nicole Levine, MD[2]; Elizabeth Sachs, MS[3]; Ashley Scholl, MD, MSc[2]; Roy Colglazier, MD[2]; Robert French, MD[2]; Rami Al-Rohil, MBBS[2]; Brian Brigman, MD, PhD[3]; William Eward, MD, DVM[3]; Julia Visgauss, MD[3], (1) Duke University School of Medicine, Durham, NC, (2) Duke University Hospital, Durham, NC, (3) Duke University, Durham, NC

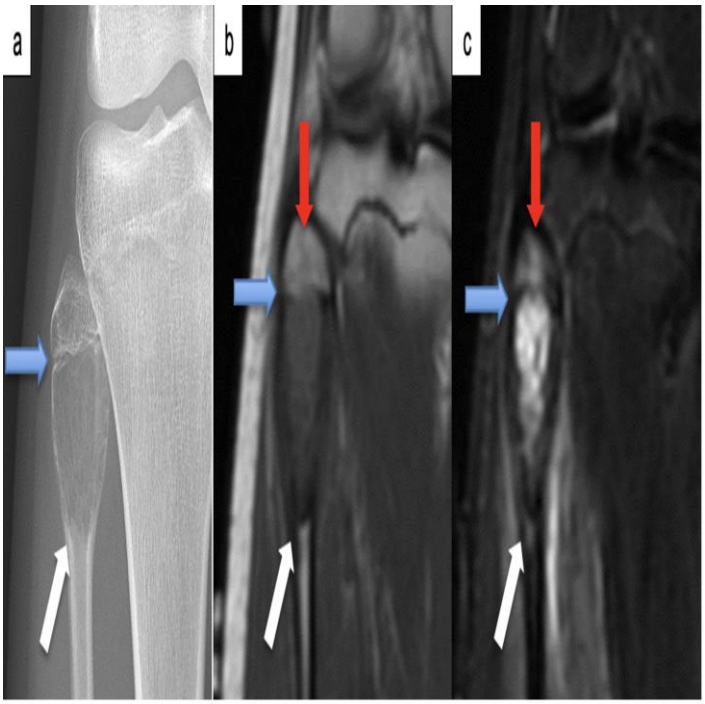
Objective: To describe the presentation of giant cell tumors of bone (GCT) in the pediatric population to 1) improve the differential diagnosis of pediatric bone tumors and 2) identify the origin of GCT. Understanding the origin of bone tumors assists in establishing appropriate diagnoses and recommending treatment options. This is particularly important in children, where evaluating the need for invasive procedures is balanced with the desire to avoid overtreatment. GCT have historically been considered epiphyseal lesions with potential metaphyseal extension. Therefore, GCT may be inappropriately excluded from the differential diagnosis of metaphyseal lesions in the skeletally immature.

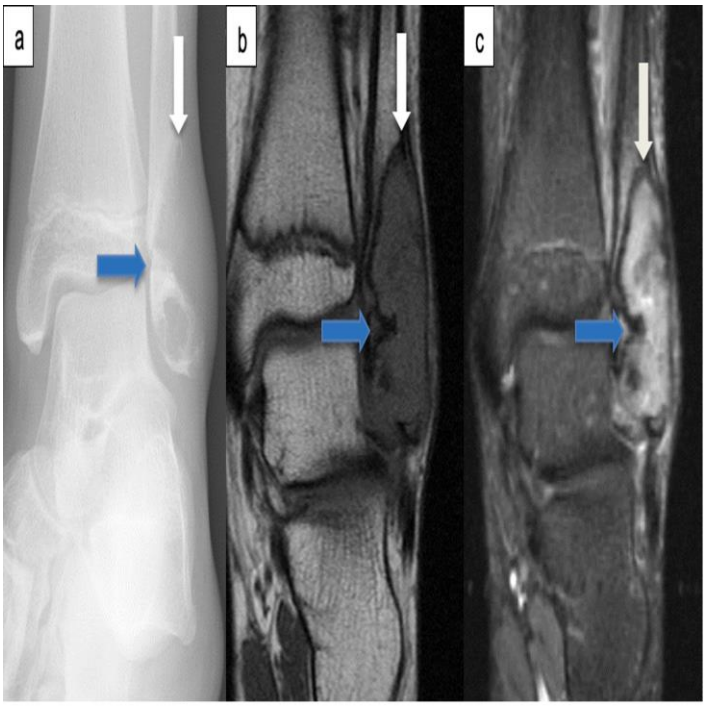
Methods: We identified 14 patients from 1981- 2021 at a single institution who had histologic confirmation of GCT and were less than 18 years old at diagnosis. Patient characteristics, tumor location, surgical treatment, and local recurrence rates were collected.

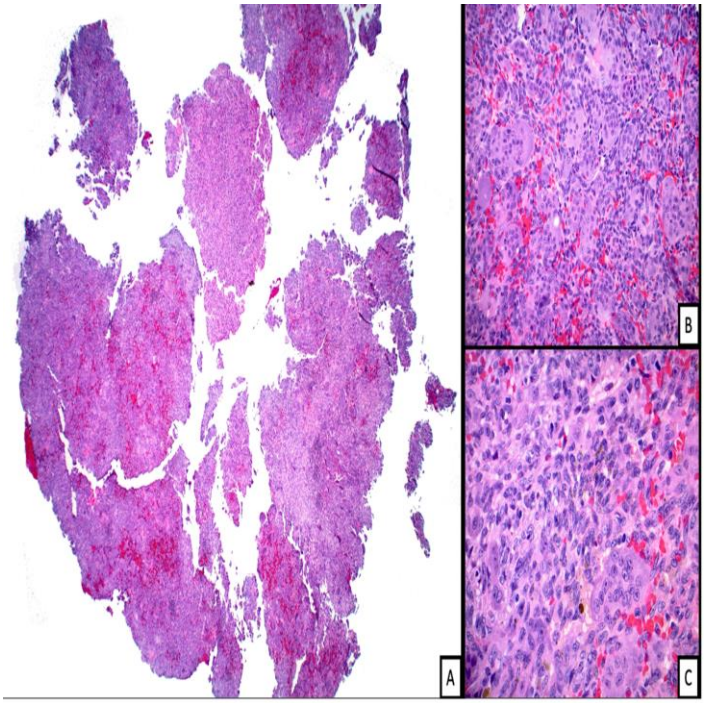
Results: Ten (71%) patients were female. Eleven (78.6%) were epiphysiometaphyseal (1 epiphyseal, 4 metaphyseal, 6 epiphysiometaphyseal). Five patients had an open adjacent physis, of which three (60%) had tumors confined solely to the metaphysis. Of the five patients with open physis, four (80%) developed local recurrence while only one patient (11%) with a

closed physis had local recurrence (p value = 0.0023).

Conclusion: Our results illustrate that for the skeletally immature, GCT can (and in our results more commonly did) occur in the metaphyseal location. These findings suggest that GCT should be included in the differential diagnosis of primary metaphyseal only lesions in the skeletally immature.







(P 510) PRIMARY BREAST ANGIOSARCOMA: CLINICAL AND PATHOLOGICAL FEATURES AND IMPACT ON OUTCOMES

Heather A. Lillemoe, MD[1]; Kelly Hunt, MD[2]; Aparna Subramaniam, MBBS, MPH[2]; Emily Z. Keung, MD, AM, FACS[2]; Christina Roland, MD, MS[2]; Keila E. Torres, MD, PhD, MBA, FACS[2]; Heather Lyu, MD[3]; Christopher Scally, MD[2]; Devarati Mitra, MD PhD[3]; Ahsan S. Farooqi, MD PhD[4]; Andrew J. Bishop, MD[3]; B Ashleigh Guadagnolo, MD,MPH[2]; Vinod Ravi, MD, MS, MBA[2], (1) University of Texas MD Anderson Cancer Center, Houston, TX, (2) The University of Texas MD Anderson Cancer Center, Houston, TX, (3) MD Anderson Cancer Center, Houston, TX, (4) MD Anderson Cancer Center, Sugar Land, TX

Objective: Primary angiosarcoma of the breast is a rare and aggressive malignancy with a propensity for metastasis and early recurrence. There are limited data related to outcomes for this disease and the data that exist are often combined with secondary angiosarcomas of the breast. The aim of this study is to characterize a primary breast angiosarcoma patient cohort at a comprehensive cancer center and assess differences in survival based on patient and treatment factors to inform future management.

Methods: In a retrospective study, patients with a diagnosis of primary breast angiosarcoma who received care at a large tertiary cancer center from 1991 through 2021 were reviewed. Patients were excluded if they had insufficient data or short follow-up time (< 3 months). Patients with a prior history of cancer requiring radiation to the chest were also excluded. All patients underwent surgical resection. Data were collected and analyzed using descriptive statistics and survival analysis was performed.

Results: Of 115 total patients, 97 were eligible for inclusion (Table 1). Median patient age was 40 years (range, 15 to 70 years) and 99% were female. Median tumor size was 6 cm (IQR 4, 8) and 59% were high grade. Most patients (81%) presented with a palpable breast mass with 13% presenting with skin changes such as rash, erythema, or bruising. There were 88 patients (91%) who received multimodality treatment: chemotherapy + surgery (n=32, 36%), radiation (RT) + surgery (n=14, 16%), or chemotherapy + RT + surgery (n=42, 48%). Most patients (n=66, 68%) were referred to our institution after surgery at an outside facility. The majority underwent total mastectomy (87%), with axillary assessment undertaken in 43%. Only 4 patients (4%) had metastatic disease in the nodes. Median follow-up time was 3.4 years (IQR 1.9, 6.2) with a median overall survival (OS) of 6.3 years (IQR 3, 12.5) and 5-year OS of 56%. Sixty-one percent of patients experienced recurrence with a median recurrence-free survival (RFS) of 1.8 years (IQR 1.1, 3.3). Most recurrences were distant only (n=42, 73%), commonly to bone or to multiple sites. Nine patients (16%) had local recurrence; 6 (10%) had recurrence and both local and distant sites. Patients with tumor size >5 cm had lower median OS compared to those with smaller tumors (4 vs. 11 years, $p< 0.001$) and those with high grade tumors had lower median OS compared to those with low (4.0 vs. 8.7 years, $p=0.004$) and intermediate grade tumors (4.0 vs. 6.3 years, $p=0.103$; Figure 1). Larger tumor size and high grade were both independently predictive of OS (Table 2), but not RFS. When stratified by decade of disease diagnosis and treatment, those treated in the earlier cohort (1991-2000) had lower median OS (Figure 2). On sub analysis of 42 patients with large tumors and high/intermediate grade, there was no difference in OS when comparing those who received trimodal treatment (n=23) to single/dual modality treatment (n=19; 3.5 vs. 4.1 years, $p=0.491$).

Conclusion: Most patients with primary breast angiosarcoma present at a young age with large, high grade tumors, which have poorer oncologic outcomes. Nodal positivity is rare. The majority of patients received multimodal therapy including a combination of surgical resection, chemotherapy, and radiation, however the impact of treatment modality on survival outcomes is unclear. Recurrence is common and often occurs within 2 years of diagnosis. Further research with defined treatment sequencing is needed to improve oncologic outcomes.

Table 1. Clinicopathologic characteristics of patients with primary breast angiosarcoma

Characteristic	Total
No. of patients	97
Age, median (IQR), years	40 (32, 48)
Sex, female	96 (99)
Tumor size, median (IQR), cm*	6 (4, 8)
Tumor grade*	
High	49 (59)
Intermediate	19 (23)
Low	15 (18)
N stage (positive)	4 (4)
M stage (positive)	4 (4)
Treatment modalities used	
Chemotherapy	74 (76)
Radiation	56 (58)
Surgery	97 (100)
Multimodality treatment (n=88)	
Chemotherapy + Surgery	32 (36)
Radiation + Surgery	14 (16)
Chemotherapy + Radiation + Surgery	42 (48)
Surgical treatment (n=97)	97 (100)
Total mastectomy	85 (87)
Breast-conserving therapy (wide local excision)	12 (12)
Axillary surgery	40 (43)
ALND	16 (40)
SLNB	24 (60)
Margins, positive*	14 (16)

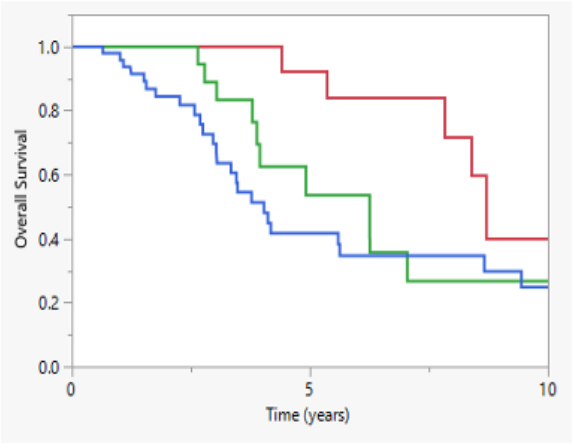
IQR, interquartile range; Values are number of patients (percentage) unless otherwise indicated.
* Missing data (percents shown for these factors include only those with data)
Size (prior to treatment): 8
Grade at presentation/bx: 14
Margins: 9
Path Grade: 23

Table 2. Univariable and multivariable analysis of overall survival

Variable	N (%)	5-year survival rate, %	Univariable P value	Hazard ratio	95% confidence interval	Multivariable P value
All patients	97 (100)	56				
Tumor factors*						
Age > 50 years	20 (21)	69	0.075			
Grade**, high	49 (59)	42	**0.003**	2.4	1.2,4.7	**0.015**
Margin, positive	14 (16)	41	0.278			
Tumor size**, >5 cm	56 (63)	43	**<0.001**	5.1	2.1,12.6	**0.002**
Treatment factors						
Surgery only	9 (9)	60	0.951			
Surgery + Radiation	14 (14)	62				
Surgery + Chemotherapy	32 (33)	48				
Triple modality therapy	42 (43)	58				

* Missing data (patients with missing data not included in analysis):
Grade: 14
Margins: 9
Tumor size: 8
**With discrepancy between grade on final pathology and pathology at diagnosis, highest grade was used. With discrepancy between size on final pathology and at diagnosis, largest size was used.

Figure 1. Overall survival based on grade



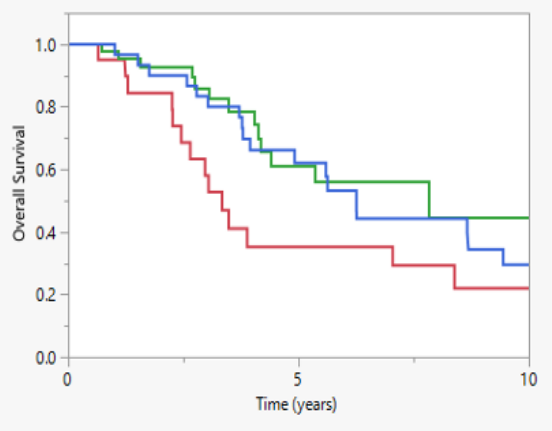
p = 0.005

Red – Low grade, median survival 8.7 years (7.8, NR); n=15

Green – Intermediate grade, median survival 6.3 years (3.9, 12.5); n=19

Blue – High grade, median survival was 4 years (2.8, 9.4); n=49

Figure 2. Overall survival trends over time



Median OS 3.3 years (early) vs. 6.3 years (mid) vs. 7.8 years (late), **P = 0.029**

No. at risk

Early (1991-2000)	20	7	4
Mid (2001-2010)	31	16	6
Late (2011-2021)	46	13	2

(P 511) SIGNIFICANCE OF TIP TO APEX DISTANCE IN PATHOLOGICAL AND IMPENDING PATHOLOGICAL FRACTURES OF THE PROXIMAL FEMUR

Matthew D. Nester[1]; Thien Huong Huynh[2]; Davis Kuruvilla, BS[3]; Odion Binitie, MD[4]; David Joyce, MD[4]; Alexander Lazarides, MD[5], (1) University of South Florida Morsani College of Medicine, Tampa, FL, (2) University of South Florida Morsani College of Medicine, Clearwater, FL, (3) University of South Florida Morsani College of Medicine, Las Vegas, NV, (4) H. Lee Moffitt Cancer Center, Tampa, FL, (5) Moffitt Cancer Center, Tampa, FL

Objective: The tip-apex distance (TAD) is a metric that accounts for the position and depth of a screw in the femoral head and has been shown to be associated with risk of screw cut-out. Traditionally, TAD > 25 mm is correlated with high failure rates. Application of this metric to pathological and/or impending pathological fractures of the proximal femur in cancer patients is lacking. The aim of this study is to assess whether a high TAD correlates with failure rate in pathological and/or impending pathological proximal femur fractures.

Methods: A retrospective chart review was conducted on all patients who underwent intramedullary nailing (IM) of the proximal femur for pathological and/or impending pathological fractures from 2007-2023 (n=205). Patients who did not have postoperative radiographs were excluded (n=71). The 134 patients that remained following exclusion criteria consisted of 21 pathological fractures and 113 impending pathological fractures. Demographics, preoperative comorbidities, TAD, complications, device failure and reoperation rates were recorded.

Results: Failure was observed in 8/134 (6%) patients. Preoperative lesion presentation of the femurs that failed fixation included 1 complete fracture and 7 impending fractures. The mode of device failure included 4 progressions of the fracture at the original fracture site, 1 nonunion, 2 failed distal locking screws and 1 due to bending of the proximal aspect of the IM rod at the level of the lag screw. Average TAD in devices that failed was 37±10 mm, whereas the average TAD in devices that did not fail was 30±10 mm, which was not statistically significant (p=0.068).

Conclusion: The results presented here suggest that TAD may not matter in appropriately selected patients, as all the hardware failures happened at the site of the lesion or distally at the interlocking screws. Furthermore, TAD was not significantly different between devices that failed and those that did not. Given the robust correlation of TAD with cut out failure in non-pathological hip fractures, further investigation into TAD in pathological femur fractures with a larger patient population is warranted.

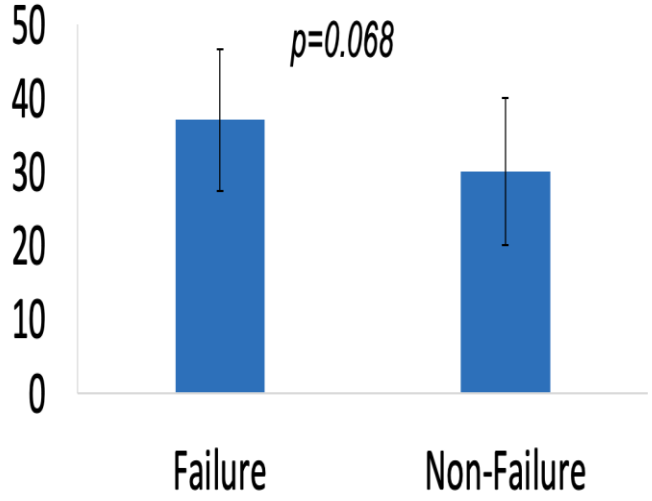


Figure 1. Average TAD (mm) with standard deviation in devices that failed (left) and those that did not fail (right).

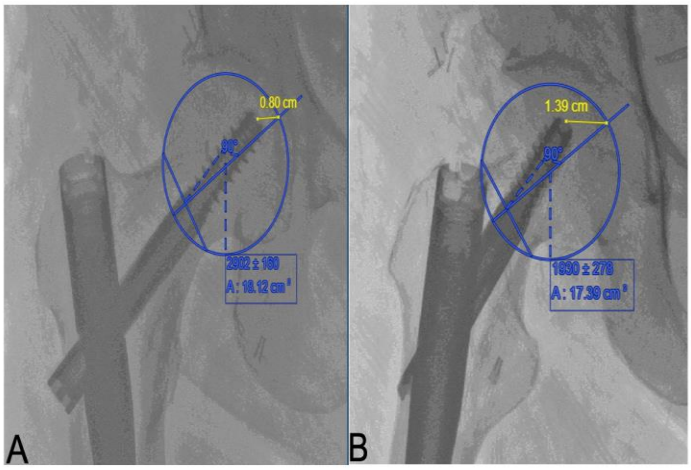


Figure 2. (A) AP and (B) lateral fluoroscopic view of hip and screw prosthesis in situ. Using the ellipse region of interest tool, an ellipse is stretched to exactly match the line of subchondral bone of the femoral head. A line is then drawn between the points where the ellipse meets the neck of the femur. Using the angle measuring tool, a line perpendicular to this line arising from its mid-point is taken through the femoral head. Where this perpendicular line bisects the ellipse defines the apex of the femoral head. Once constructed, the linear measuring tool is used to determine the distance between the tip of the lag screw and the apex of the femoral head. TAD is then calculated as the some of the AP and lateral values.

(P 512) PERIOPERATIVE BLOOD MANAGEMENT IN PEDIATRIC PATIENTS UNDERGOING PRIMARY OSTEOSARCOMA AND EWING SARCOMA RESECTION: A RETROSPECTIVE CASE NOTE REVIEW

Mary-Claire Simmonds, MBBS, FANZCA[1]; Laveniya Kugathasan, BSc Pharmacology[2]; Jeffrey Bone, MSc[3]; Nicholas West, BSc, MSc[3]; Steffanie Fisher[4]; Paul Clarkson, MBChB, FRACS, FRCSC, MSc[1]; Shahrad Rod Rassekh, MD, MHSc[5]; Teresa Skelton, MD, FRCPC, MPH[6], (1) British Columbia Children's Hospital, Vancouver, BC, Canada, (2) University of British Columbia, Vancouver, BC, Canada, (3) British Columbia Children's Hospital Research Institute, Vancouver, BC, Canada, (4) British Columbia Children's Hospital, (5) British Columbia Children's Hospital, University of British Columbia, Vancouver, BC, Canada, (6) BC Children's Hospital, University of British Columbia, North Vancouver, BC, Canada

Objective: Resection of osteosarcoma and Ewing sarcoma constitutes major surgery and can be associated with significant blood loss [1,2]. Along with the high incidence of preoperative anemia in cancer patients due to neoadjuvant therapy, this means allogeneic red blood cell (RBC) transfusion is often needed perioperatively. Blood transfusion can cause significant changes in the immune response of the recipient (transfusion related immunomodulation) [3], which is associated with infection and may increase recurrence in some cancers [4,5]. Intraoperative blood conservation strategies, such as cell salvage, may improve the quality of care for patients undergoing sarcoma resection by reducing or eliminating the need for allogeneic RBC transfusion.

We aimed to evaluate perioperative blood management and transfusion practice in children undergoing sarcoma resection in our tertiary pediatric centre and identify opportunities for quality improvement (QI).

Methods: We conducted a retrospective review of all primary osteosarcoma and Ewing sarcoma resection surgeries that occurred in children aged 0-18 years between January 2005 and September 2021 at British Columbia Children’s Hospital, Vancouver, Canada. There were no exclusion criteria. As a QI study, our institutional research ethics board waived the requirement for ethical review. Data collection via manual case note review focused on patient demographics, tumour and procedure details, volume and timing of transfusion, perioperative hemaglobin (Hb), and any complications related to transfusion during the perioperative period (up to 72 hours post-surgery). Anemia was defined as Hb < 120 g/L.

Primary outcomes included the proportion of patients who received an allogeneic RBC transfusion and the volume of RBCs administered (mL/kg). Secondary outcomes included proportion of patients with preoperative anemia, pre- and postoperative Hb level, and complication rates associated with RBC transfusion. Generalized linear models were used to evaluate factors associated with increased perioperative allogeneic RBC transfusion.

Results: A total of 72 patients of median [interquartile range (IQR)] age 14 [11; 15] were included. Demographic data were similar between patients that received an RBC transfusion vs. those that did not (Table 1).

Preoperatively, 59/70 (84%) patients were anemic, with a median [IQR] Hb of 108 g/L [99; 117]; all patients (59/59) were anemic postoperatively, with a day one median Hb of 90 g/L [80; 102].

An RBC transfusion was given to 45/72 (63%) patients intraoperatively, with a median [IQR]

volume of 608 mL [424; 1096], and to 28/72 (39%) patients postoperatively, with a volume of 558 mL [303; 892] (Table 2).

An RBC transfusion was given in all seven cases with a pelvic tumour. Tumour in the pelvis was associated with an increased volume of RBC transfusion intraoperatively with a mean difference [MD] of 30.9 mL/kg (95%CI 20.7 to 41.2). Other factors associated with an increased volume of RBC transfusion perioperatively included presence of metastatic disease at time of surgery (MD 3.4 mL/kg, 95%CI 0.5 to 6.3) and tumour in the upper leg (MD 3.1 mL/kg, 95%CI 0 to 6.2) (Table 3).

In patients that received an RBC transfusion postoperatively, 3/26 (12%) experienced a transfusion-related complication: two experienced fever up to 39°C and one developed fever and subjective chills.

Conclusion: This retrospective review suggests that perioperative anemia is common in patients undergoing primary resection of osteosarcoma or Ewing sarcoma. Perioperative allogeneic RBC transfusion is often required, especially in patients with existing metastatic disease or with a pelvic tumour. This was associated with transfusion related complications. Avoiding allogeneic RBC transfusion may be beneficial in some cases. Further research into potential benefits and safety of blood conservation strategies, such as intraoperative cell salvage, may be warranted in this patient population.

Table 1: Cohort demographics divided by cases with or without a perioperative allogeneic red blood cell (RBC) transfusion

	No RBC transfusion (N=21)	**RBC transfusion** (N=51)
Age (years)	12.5 [9.0; 14.8]	14.3 [11.1; 15.9]
Sex (Female; Male)	12; 9 (57%; 43%)	25; 26 (49%; 51%)
Weight (kg)	50.3 [30.8; 61.2]	45.5 [36.8; 58.4]
Height (cm)	159 [143; 170]	159 [147; 167]
ASA Physical Status		
I – II	5 (24%)	10 (20%)
III – IV	15 (71%)	31 (61%)
Not recorded	1 (5%)	10 (20%)
Surgery		
Ewing sarcoma resection	9 (43%)	16 (31%)
Primary osteosarcoma resection	12 (57%)	35 (69%)
Histological grade		
3	2/11 (18%)	5/25 (20%)
other	9/11 (82%)	20/25 (80%)
Presence of metastatic disease	2 (10%)	**13 (27%)**
Location of Tumour		
upper arm	2 (10%)	5 (10%)
upper leg	2 (10%)	26 (51%)
lower leg	9 (43%)	9 (18%)
pelvis	0	7 (14%)
spine	1 (5%)	1 (2%)
other	7 (33%)	3 (6%)

Data are presented as median (interquartile range) for continuous variables and count (%) for categorical variables; ASA = American Society of Anesthesiologists

Table 2: Allogeneic red blood cell (RBC) transfusion details

	Intraoperative RBC transfusion (N=45)	**Postoperative** RBC transfusion (N=28)
Volume of RBC transfused (mL)	608 [424; 1096]	558 [303; 892]
Volume of RBC by patient weight (mL/kg)	13.1 [9.9; 27.9]	11.8 [8.5; 18.1]
Units transfused		
1 – 2 units	25 (55%)	19 (68%)
> 2 units	20 (45%)	9 (32%)

Data are presented as median (interquartile range) for continuous variables and count (%) for categorical variables

Table 3: Predictors of total allogeneic red blood cell (RBC) transfusion volume/kg

Factor	**Intraoperative** RBC transfusion	**Perioperative** RBC transfusion
Age (years)	0 (-0.17, 0.17)	0.02 (-0.04, 0.08)
Weight (kg)	-0.03 (-0.21, 0.15)	0 (-0.06, 0.07)
Type of surgery: primary osteosarcoma resection	-2.73 (-9.93, 4.47)	0.49 (-2.13, 3.1)
Presence of metastatic disease	7.88 (-0.27, 16.03)	**3.4 (0.48, 6.32)**
Pre-operative Hb Level	0.06 (-0.32, 0.43)	-0.05 (-0.19, 0.08)
Intraoperative administration of tranexamic acid (TXA)	6.27 (-0.52, 13.06)	1.31 (-1.18, 3.81)
Tumour located in pelvis	**30.9 (20.66, 41.15)**	2.1 (-2.46, 6.66)
Tumour located in upper leg	9.05 (2.1, 16)	**3.1 (0, 6.19)**

Data are presented as **mean difference** (with 95% confidence intervals). All analyses were univariate due to small sample sizes and includes patients with no transfusion (volume transfused set to 0).

References

1. Taran SJ, Taran R, Mlipatil, NB. *Pediatric Osteosarcoma: An Updated Review*. Indian Journal of Paediatric Oncology. (2017) 38(1): 33-043
2. Tang X, Guo W, Yang R, Tang S, Ji T. *Evaluation of blood loss during limb salvage surgery for pelvic tumours.* Int Orthop (2009) 33:3 pp751-756
3. Pandey P, Chandhary R, Aggarwal A, Kumar R, Khetan D, Verma A. *Transfusion associated immunomodulation: quantitative changes in cytokines as a measure of immune responsiveness after one time blood transfusion in neurosurgery patients.* Asian J Transfus Sci (2010) 4:2 pp 78-85.
4. Blumberg N, Heal JM. *Effects of transfusion on immune function-cancer recurrence and infection*. Arch Pathol Lab Med. 1994;118:371–9
5. Heiss MM, Mempel W, Delanoff C, Jauch KW, Gabka C, Mempel M, et al. *Blood transfusion modulated tumor recurrence: First results of a randomized study of autologous versus allogeneic blood transfusion in colorectal cancer surgery.* J Clin Oncol. 1994;12:1859–67

(P 513) THE IMPACT OF MARGIN STATUS ON THE ONCOLOGIC OUTCOMES OF SURGICALLY TREATED DEDIFFERENTIATED LIPOSARCOMA OF THE EXTREMITIES

Lukas M. Nystrom, MD[1]; Martina Hale, BA[2]; Precious C. Oyem, BA[1]; Zachary D. Burke, MD[3]; Nathan W. Mesko, MD[1]; Scott E. Kilpatrick, MD[1], (1) Cleveland Clinic, Cleveland, OH, (2) Cleveland Clinic Lerner College of Medicine, Cleveland, OH, (3) Celveland Clinic Sarcoma Program, Cleveland, OH

Objective: Dedifferentiated liposarcoma (DDLPS) is an aggressive variant of liposarcoma that can arise within an atypical lipomatous tumor (ALT). The primary goal of treatment for DDLPS is wide margin resection, but there is sparse data to suggest whether the low-grade portion surrounding DDLPS also necessitates wide margin resection for optimal local control. We aimed to investigate the importance of surgical marginal location with respect to DDLPS and ALT tumor portions. The behavior of ALT/DDLPS at the margin can be rather unique in its ability to insinuate itself between tissue planes. This makes the achievement of a truly “wide” margin a difficult, if not impossible endeavor, in limb salvage surgery. This study aims to evaluate the oncologic outcomes of DDLPS with respect to the margins achieved at the time of surgical resection.

Methods: This retrospective institutional cohort study included all patients treated surgically for histologically confirmed DDLPS from 2007 to 2019. Patient and tumor demographics and oncologic outcomes were collected. Exclusion criteria included patients with less than two years of follow-up, intraperitoneal/retroperitoneal or head/neck liposarcomas, or age younger than 18 years. Pathology slides were re-reviewed by a surgical pathologist specializing in soft-tissue sarcoma. Margin status was categorized into one of the following categories as described by Enneking in the surgical staging system: wide (R0), marginal (R1) and intralesional (R2). These

descriptors were applied to both the DDLPS and the ALT portions of the tumor. All patients were determined as either “wide throughout” or “wide on DDLPS, marginal on ALT”. Statistical analysis for patient outcome and margin status was carried out using chi-square tests for each patient outcome.

Results: 22 patients underwent oncologic resection for extremity DDLPS. 14 (63.6%) patients had surgical margins that were defined as wide on DDLPS/marginal on ALT, and 6 (27.3%) patients had surgical margins wide throughout, and 2 were unable to be determined (Table 1). The overall rate of local control was 90.9 % (20 of 22). 2 patients with surgical margins wide on DDLPS/marginal on ALT developed local recurrence compared with no patients with margins wide throughout (p = .53)(Table 2). 6 patients with surgical margins wide on DDLPS/marginal on ALT developed metastases compared to 1 patient with margins wide throughout (p = .31).

Conclusion: The surgical goal in DDLPS resection, as with other sarcomas, is a wide surgical resection. The data presented here do not alter that. However, this may not be feasible in many limb sparing surgeries. Our data indicate that wide compared with marginal resection of the surrounding low-grade portion did not appear to influence local recurrence, development of metastatic disease, or death from disease. However, due to the small size of this cohort, further investigation and discussion of margin importance is necessary. This study lays the foundation for future larger scale collaborative efforts to further determine the importance of margin acquisition as it relates to the ALT component of DDLPS.

Table 1: Patient Demographics and Baseline Characteristics Stratified by Presence or Absence of New Metastasis

	No Metastasis (N=16)	Metastasis (N=6)	Total (N=22)	p value
Age at diagnosis (Mean (SD))	68.12 (12.07)	60.33 (14.69)	66.00 (12.97)	0.217
Sex				0.254
Female	3 (18.8%)	0 (0.0%)	3 (13.6%)	
Male	13 (81.2%)	6 (100.0%)	19 (86.4%)	
Race				0.736
Black/African American	1 (6.2%)	1 (16.7%)	2 (9.1%)	
Multiracial/Multicultural	1 (6.2%)	0 (0.0%)	1 (4.5%)	
Unknown	1 (6.2%)	0 (0.0%)	1 (4.5%)	
White/Caucasian	13 (81.2%)	5 (83.3%)	18 (81.8%)	
BMI (Mean (SD))	28.27 (5.49)	29.43 (10.07)	28.59 (6.78)	0.73
Smoking status				0.146
Current	3 (18.8%)	2 (33.3%)	5 (22.7%)	
No	6 (37.5%)	4 (66.7%)	10 (45.5%)	
Previous Smoker	7 (43.8%)	0 (0.0%)	7 (31.8%)	
Location				0.375
Antecubital Fossa	1 (6.2%)	0 (0.0%)	1 (4.5%)	
Chest Wall	0 (0.0%)	1 (16.7%)	1 (4.5%)	
Femur/Thigh	10 (62.5%)	4 (66.7%)	14 (63.6%)	
Leg (tibia, fibula)	2 (12.5%)	1 (16.7%)	3 (13.6%)	
Shoulder Girdle (scapula, clavicle)	3 (18.8%)	0 (0.0%)	3 (13.6%)	
Extremity				0.122
Lower Extremity	12 (75.0%)	5 (83.3%)	17 (77.3%)	
Trunk/Thoracic/Abdominal Cavity	0 (0.0%)	1 (16.7%)	1 (4.5%)	
Upper Extremity	4 (25.0%)	0 (0.0%)	4 (18.2%)	
Grade				0.41
G1	1 (6.2%)	0 (0.0%)	1 (4.5%)	
G2	7 (43.8%)	1 (16.7%)	8 (36.4%)	
G3	7 (43.8%)	5 (83.3%)	12 (54.5%)	
Unknown	1 (6.2%)	0 (0.0%)	1 (4.5%)	
AJCC stage				0.725
I	2 (12.5%)	0 (0.0%)	2 (9.1%)	
II	4 (25.0%)	2 (33.3%)	6 (27.3%)	
III	9 (56.2%)	4 (66.7%)	13 (59.1%)	
Unknown	1 (6.2%)	0 (0.0%)	1 (4.5%)	
Surgical Margin Description				0.451
Unknown	2 (12.5%)	0 (0.0%)	2 (9.1%)	
Wide on dedifferentiated; marginal on ALT	9 (56.2%)	5 (83.3%)	14 (63.6%)	
Wide throughout	5 (31.2%)	1 (16.7%)	6 (27.3%)	
Local Recurrence	0 (0.0%)	2 (33.3%)	2 (9.1%)	**0.015**
Died of Disease	0 (0.0%)	4 (66.7%)	4 (18.2%)	**< 0.001**
No evidence of disease	15 (93.8%)	0 (0.0%)	15 (68.2%)	**< 0.001**
Alive with Disease	1 (6.2%)	2 (33.3%)	3 (13.6%)	0.099

Table 2: Patient Outcomes Stratified by Surgical Margin

	Wide on dedifferentiated; marginal on ALT (N=14)	Wide throughout (N=5)	Unknown (N=2)	Total (N=22)	p value
Local Recurrence	2 (14.3%)	0 (0.0%)	0 (0.0%)	2 (9.1%)	0.533
Died of Disease	3 (21.4%)	1 (16.7%)	0 (0.0%)	4 (18.2%)	0.758
No Evidence of Disease	8 (57.1%)	5 (83.3%)	2 (100.0%)	15 (68.2%)	0.308
Alive with Disease	3 (21.4%)	0 (0.0%)	0 (0.0%)	3 (13.6%)	0.371
New Metastasis	6 (42.9%)	1 (16.7%)	0 (0.0%)	7 (31.8%)	0.308

(P 514) INTERSTITIAL BRACHYTHERAPY IN THE TREATMENT OF SOFT TISSUE SARCOMA: LOCAL RECURRENCE AND WOUND OUTCOMES

Martina Hale, BA[1]; Precious C. Oyem, BA[2]; Haley Prough, DO[3]; Shauna R. Campbell, DO[4]; Jacob G. Scott, MD PhD[5]; Chirag Shah, MD[6]; Zachary D. Burke, MD[7]; Nathan W. Mesko, MD[2]; Lukas M. Nystrom, MD[2], (1) Cleveland Clinic Lerner College of Medicine, Cleveland, OH, (2) Cleveland Clinic, Cleveland, OH, (3) Cleveland Clinic Foundation, Lansing, MI, (4) Cleveland Clinic Foundation, Cleveland, OH, (5) Cleveland Clinic, Gates mills, OH, (6) Cleveland Clinic, Brecksville, OH, (7) Celveland Clinic Sarcoma Program, Cleveland, OH

Objective: Interstitial brachytherapy (BT) represents one form of delivery of adjuvant radiotherapy in the treatment of soft tissue sarcoma (STS). Compared to external beam radiation (EBRT), which is the more commonly employed technique to treat STS, BT provides a dosimetric advantage by localizing to the tumor bed and minimizing damage to peripheral healthy tissue. When employing BT, wound management options include leaving the wound open with vacuum assisted closure (VAC), allowing for confirmation of surgical margins, immediately delivering radiation therapy, or closing the wound over the catheters and delaying radiation until five days following closure. Little is known about how BT, and variations in wound management/timing, affect wound and oncologic outcomes.

Methods: This retrospective institutional cohort study included all patients receiving interstitial BT following STS resection between 2015 and 2022. Patient and tumor demographics, radiation characteristics, and wound and oncologic outcomes were collected. Statistical analysis for wound complication occurrence and reoperation were assessed using chi-squared tests. The relationship between exposure variables and wound complications was analyzed using univariate logistic regression.

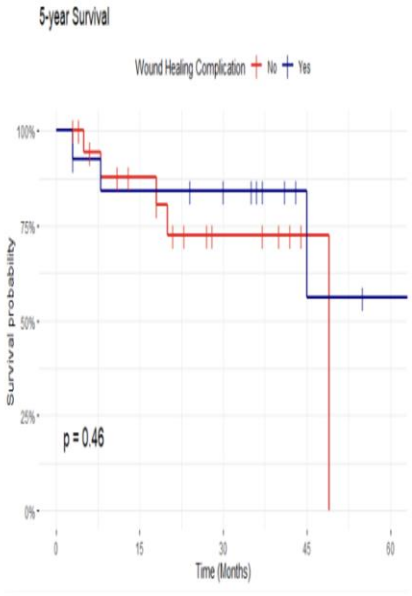
Results: 32 patients with STS of the extremity were treated with brachytherapy. 19 (59.4%) of the tumors were superficial. The mean tumor size was 5.8 cm (+/- 3.2 cm), and 30 (93.8%) were intermediate/high grade or ungraded sarcomas. 28 patients (87.5%) were treated with delayed wound closure at the conclusion of BT. This was potentiated by a VAC dressing while receiving BT (Table 1). 22 (68.8%) of the 32 patients included in the study were treated with definitive brachytherapy with a median time from biopsy to completion of brachytherapy (completion of treatment) of 43 days. Median implant size was 9 catheters, dose 38.5 Gray delivered in 10 fractions. 13 (40.6%) patients had wound healing complications with 7 (21.9%) requiring reoperation. Operative complications included necrosis (6), dehiscence (3), deep infection (1), cellulitis (1), and hematoma (1). Tumor size, BT catheter number, closure technique, and reconstruction technique were not associated with reoperation. 2 patients (6%) had a local recurrence, and 9 (28%) patients developed metastatic disease. There was no difference in overall survival for those who experienced complications compared with those who did not (Figure 1).

Conclusion: BT combined with surgery is an effective treatment to maximize local control for STS. Our results indicate BT has a similar wound complication and local control profile compared with reported values for patients treated with EBRT. BT offers the advantages of immediate tumor removal, condensed treatment duration, and minimized morbidity to the surrounding local tissues.

Table 1: Demographic and Baseline Patient Characteristics

	No Wound Healing Complications (N=19)	Wound Healing Complications (N=13)	Total (N=32)	p value
Age at Diagnosis (Mean (SD))	55.47 (19.24)	63.38 (14.23)	58.69 (17.58)	0.217
Sex				0.719
Female	9 (47.4%)	7 (53.8%)	16 (50.0%)	
Male	10 (52.6%)	6 (46.2%)	16 (50.0%)	
Race				0.051
Black/African American	1 (5.3%)	4 (30.8%)	5 (15.6%)	
White/Caucasian	18 (94.7%)	9 (69.2%)	27 (84.4%)	
BMI (Mean (SD))	29.65 (6.17)	31.76 (7.13)	30.51 (6.55)	0.381
Smoking status				0.123
Current smoker	3 (15.8%)	0 (0.0%)	3 (9.4%)	
Never a smoker	9 (47.4%)	4 (30.8%)	13 (40.6%)	
Previous smoker	7 (36.8%)	9 (69.2%)	16 (50.0%)	
Sarcoma Type				0.277
New Untreated Sarcoma	10 (52.6%)	10 (76.9%)	20 (62.5%)	
Unplanned Resection	8 (42.1%)	2 (15.4%)	10 (31.2%)	
Recurrent Sarcoma	1 (5.3%)	1 (7.7%)	2 (6.2%)	
Extremity				0.056
Lower extremity	10 (52.6%)	12 (92.3%)	22 (68.8%)	
Other	2 (10.5%)	0 (0.0%)	2 (6.2%)	
Upper Extremity	7 (36.8%)	1 (7.7%)	8 (25.0%)	
Depth				0.348
Deep	9 (47.4%)	4 (30.8%)	13 (40.6%)	
Superficial	10 (52.6%)	9 (69.2%)	19 (59.4%)	
Stage				**0.014**
Stage IA	0 (0.0%)	2 (15.4%)	2 (6.2%)	
Stage IB	1 (5.3%)	0 (0.0%)	1 (3.1%)	
Stage II	10 (52.6%)	0 (0.0%)	10 (31.2%)	
Stage IIIA	5 (26.3%)	8 (61.5%)	13 (40.6%)	
Stage IIIB	2 (10.5%)	3 (23.1%)	5 (15.6%)	
Stave IV	1 (5.3%)	0 (0.0%)	1 (3.1%)	
Mean Greatest Tumor Dimensions on Pre-Op Imaging in cm (SD)	5.41 (3.90)	6.38 (1.65)	5.81 (3.18)	0.404
Wound management				0.683
Delayed closure/VAC over implant	17 (89.5%)	11 (84.6%)	28 (87.5%)	
Immediate closure over implant	2 (10.5%)	2 (15.4%)	4 (12.5%)	

Figure 1. Kaplan-Meier 5-year survival curve for patients experiencing complications vs those without complications.



(P 515) ENDOBRONCHIAL NAVIGATION AS AN INTRAOPERATORY TOOL FOR RESECTION OF SMALL PULMONARY METASTASES IN SARCOMA PATIENTS

Elena Fernández, MD[1]; Carlos A. Fraile Olivero, MD, PhD[2]; José Ramón Jarabo, Md, PhD[2]; Verónica Alen, MD[2]; Lidia Sotillo, MD[2]; Verónica Albarrán, MD[2]; Florentino Hernando, MD, PhD[2]; Gloria Marquina, MD, PhD[2]; Arancha Manzano, MD, PhD[3]; Antonio Casado, MD[4]; María José Bernabé, MD, PhD[2]; Melchor Saiz, MD[2]; Cristina Díaz, MD, PhD[2]; Luis Ortega, MD[2]; Maria Fernanda Ollarves, MD[2]; Irene Martín, MD[2]; María Vidal, MD[2]; Paula Hernandez, MD[2]; Beatriz Cabeza, MD[2]; Ana Bustos, PhD[2]; Gonzalo Civantos, MD[2]; Ana Paula Leiva, Nurse[5], (1) Hospital Clínico San Carlos, Las Rozas de Madrid, Madrid, Spain, (2) Hospital Clínico San Carlos, Madrid, Madrid, Spain, (3) HU Clínico San Carlos, Madrid, Madrid, Spain, (4) Hospital Universitario Clínico San Carlos, Madrid, Madrid, Spain, (5) Hospital Clinico san Carlos, Madrid, Madrid, Spain

Objective: To describe our new approach combining intraoperatively electromagnetic navigation bronchoscopy (ENB) with pleural dye marking and/or technetium99-macroaggregated albumin (99mTC-MAA) administration in a series of pulmonary nodules thoracoscopically resected.

Methods: A first phase of planning was developed by reconstruction of a computed tomography (CT) (thickness of 1-1.25 mm; overlap 20-50%) with the Illumisite© version 7.2 of Medtronic. We obtained a 3D image of the tracheobronchial tree which was used to create a pathway to reach the target (pulmonary nodule). The second phase was carried out directly at the operating room under general anesthesia. An electromagnetic field was generated by placing a board under the thorax of the patient combined with three electrodes. Then we performed a bronchoscopy with a locatable guide provided with an electrode to guide us through that field. Once reached the target, we administered 12MBq (0.2 ml) of 99mTC-MAA or

0.1 ml of the chosen dye (indocyanine green or methylene blue). The intrapulmonary deposit was confirmed with a portable gammacamera in the case of 99mTC-MAA and the activity measured in vivo. Finally, a video-assisted thoracoscopic surgery (VATS) was carried out. We used a gamma detector probe to localize the pulmonary region of maximum activity (hotspot) of 99mTC-MAA and a wedge resection was performed. In cases of dye marking, we localized directly the pleural surface coloured in green or in blue. We analyzed variables related to demography, navigation, surgery, postoperatory and histology. We considered a successful procedure when the central allocation of the lesion was situated within 1.5 cm of the hotspot or of the dye marking in the specimen.

Results: From 1st February 2022 to 31st March 2023 we localized 19 pulmonary nodules (mean size 9.4 mm, SD 4.2) in 15 patients combining ENB with 99mTC-MAA and/or dye marking. Mean time of ENB procedure was 16 minutes (SD 8.7). Mean time required to localize the hotspot intraoperatively was 1 minute (1-3) in cases of 99mTC-MAA. Successful rate was 84.2%. Four of the 19 nodules resected (21.1%) corresponded to sarcoma metastases (leiomyosarcoma, osteosarcoma and rhabdomyosarcoma; mean size 6.8 mm, SD 1.92), being all margins of resection microscopically free from tumour. (See Table 1).

Conclusion: Combining ENB with 99mTC-MAA or dye marking allowed us to localize small and peripheral pulmonary lesions, otherwise difficult to palpate by VATS. In our experience, it is a feasible, quick and reproducible technique, with a low rate of easily solved complications. Combination of BNE with 99mTC-MAA is a new option, not previously described, for localizing pulmonary nodules. Given the high incidence of pulmonary metastases from sarcomatous tumours during the evolution of the disease (in many cases small nodules difficult to palpate through minimally invasive approaches), we should have in mind BNE as a useful tool in cases of selected metastasectomy.

Demograghic and pulmonary lesion variables	
-Gender	Male 66.7%
-Age	60.7 years (SD 15.6)
-Location	RLL 26.3% LUL 26.3% LLL 21.1%
-Type of pulmonary nodule	Solid 57.9% Part-solid 31.6% Ground glass opacity 5.3%
-Distance to pleural surface	10.9 mm (SD 9.5)
Navigation and intraoperative variables	
-Distance to target in ENB	10.8 mm (SD 0.5)
-Complications during ENB	13.3% (pneumothorax)
-Duration of navigation procedure	16 minutes (SD 8.7)
-Type of marking	^{99m}TC-MAA 60% Indocyanine green 20% Indocyanine green + ^{99m}TC-MAA 13.3% Methylene blue 6.7%
-Distance within lesion and hotspot in specimen	6.9 mm (SD 7)
Postoperative and specimen variables	
-Length of hospital stay	2.5 days (SD 0.8)
-Postoperative complications	13.3% (atrial arrhythmia and subcutaneous emphysema)
-Lesion resection margin	10.1 mm (SD 3.8)
-Final pathology report	Pulmonary metastases 52.6% (sarcoma 21.1%) Lung adenocarcinoma 31.6% Benign 15.8%

Table 1: Results

(P 516) **OUCOMES OF AGGRESSIVE RESECTION OF RADIATION INDUCED ANGIOSARCOMA OF THE BREAST**

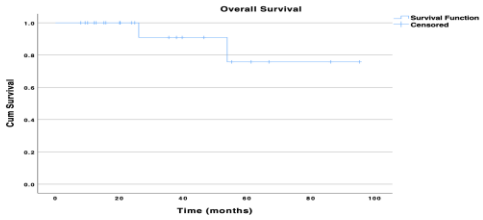
Eyal Mor, MD[1]; Benjamin Baker, MBA FRCS(Plast)[2]; Michael A. Henderson, MD FRACS[2]; David Speakman, MD[2]; Richard Zinn, MD[2]; Angela Webb, MD[2]; Hayden Snow, MD[2]; David E Gyorki, MD[2], (1) Peter MacCallum Cancer Center, Parkville, Victoria, Australia, (2) Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

Objective: Radiation induced angiosarcoma (RIAS) of the breast is a rare tumor with high rate of local recurrence. Current acceptable treatment is surgical resection in cases of localized disease. There are no strict guidelines regarding the extent of resection. Recent studies have showed that a more extensive or radical resection may reduce the rate of local recurrence. The aim of this study is to evaluate the outcome of radical resection performed at our center.

Methods: A retrospective analysis of all patients who underwent surgical resection for RIAS of the breast between 2007-2022 at a tertiary academic referral center (n=26). Included were patients who have localised disease to the breast (no metastatic disease on staging) and underwent radical resection, includes complete resection of the majority of previously irradiated skin, subcutaneous tissue and underlying fascia of pectoralis major (or muscle resection depending on extent of deep tumour invasion) Post-operative and long-term oncological outcomes were than analyzed.

Results: Twenty-two (n=22) patients met the inclusion criteria. Median age was 73.5 (range, 62-85). Median time from radiation to diagnosis of angiosarcoma was 106 months (range, 50.1-343). The median Charlson comorbidity index was 6 (range 1-8). Of all patients, 5 (22.2%) had their pectoralis fascia removed, 7 (31.8%) had both pectoralis fascia and part of the underlying muscle and 9 (40%) had complete pectoralis major muscle removed during surgery. 4 (18%) patients had reconstruction with local flaps, 12 (54%) with a pedicle flap, and 6 (27%) with a free flap. The rate of post-operative complication was 13.6% (n=3) in which only 1 (45) of them was major and was related to the reconstruction. The mean size of the specimen was 201mm (±58) with a mean size of tumor of 52mm (±27). Most of the patients had a high-grade tumor (grade II in 40%, and grade III in 50%), with 17 patients had a single lesion (77.2%) and 5 (22.7%) with a multifocal disease. One (4.5%) patient received neoadjuvant therapy while 2 (9%) patients received adjuvant therapy. At a median follow-up of 25.4 months (range, 7.9-95.2), 3 (13.6%) patients had both local and metastatic lung disease and 1 (4%) patient with only lung metastasis. For those patients who recurred, the median time to recurrence was 19.2 months (range, 4.6-24.3). The estimated 3 and 5-year OS was 90.9% and 75.7% respectively, with a median that was not reached (mean 82.6 months). The estimated 3- and 5-year disease free survival rate are both 75.1% with a median that was not reached (mean 78.6 months).

Conclusion: Treatment of RIAS of the breast with an up-front locally aggressive approach is associated with a low rate of local recurrence.



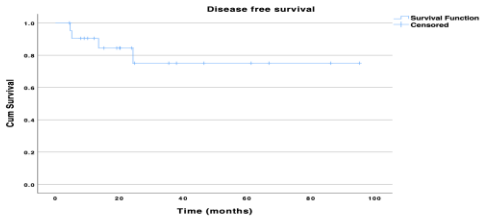


Table 1

Variable	*n*=22	*value*
Median age in years, (range)	73.5	62-85
Sex		
Female, *n* (%)	22	100%
ASA score,		
median (range)	2	2-3
II, *n* (%)	13	59%
III, *n* (%)	9	41%
Charlson comorbidity index (CCI)		
median (range)	6	1-8
Time from breast to angiosarcoma		
median months (range)	108	51-345
Type of breast cancer		
IDC	20	90%
ILC	2	10%
Type of breast surgery		
Lumpectomy, *n* (%)	20	90%
Mastectomy, *n* (%)	2	10%
Sentinel lymph node biopsy, *n* (%)	8	36%
Axillary lymph node dissection, *n* (%)	14	64%
Breast cancer treatment		
Adjuvant radiotherapy, *n* (%)	22	100%
Adjuvant chemotherapy, *n* (%)	15	68.1%
Adjuvant hormone therapy, *n* (%)	12	54.5%
Radiation dosage, median (range)	50	42-50

Table 2

Variable	*n*=22	*value*
Resection		
Pectoralis major fascia, *n* (%)	5	22.2%
Partial muscle resection, *n* (%)	7	31.8%
Complete muscle resection, *n* (%)	9	40%
Post-operative complications		
Total, *n* (%)	3	13.6%
Majors, *n* (%)	1	4%
Path:		
Resection margins:		
R0	22	100%
Size of the specimen (mm), mean (±SD)	201.5	58.9
Size of the tumor (mm), mean (±SD)	52	27.5
Grade		
I, *n* (%)	2	9%
II, *n* (%)	9	40%
III, *n* (%)	11	50%
Tumor invasion		
Skin/subcutaneous, *n* (%)	12	54.5%
Underlying fat/breast tissue, *n* (%)	4	18.8%
Muscle, *n* (%)	6	27.2%
Focality		
Single lesion, *n* (%)	17	77.2%
Multi-focal, *n* (%)	5	22.7%
Site of recurrence		
Local	3	13.6%
Distant	4	18.1%

Name	Patients (n)	Median age years (range)	Surgery	Focality (S, MF*)	Complications	Median follow-up (months)	Recurrences (n)	Metastasis (n)	OS	DFS/DSS
Li et al (2017)	76 38 radical 38 conservative	65 (57–69)	38 (50%) Radical	80% MF	44% total 16% Major	27m (IQR 11 – 60)	32 (42%) 6 (15%) of radical 26 (68%) conservative	19 (25%) 5 (13.1%) of radical 14 (375) conservative	n/a	5Y DSS 86%
Erel et al (2010)	25 (12 breast – 5 angiosarcoma)	51 (33-79)	Conservative/Unknown	n/a	n/a	n/a	11 (52%)	10 (40%)	5Y-27%	n/a
Fodor et al (2006)	8	72 (61-80)	Radical mastectomy	4 (50%) S 4 (50%) MF	n/a	n/a	8 (100%)	5 (62.5%)	Mean 26m. 2Y – 18%	2Y – 19%
Billing et al (2004)	27	69 (50-83)	9 (33%) Mastectomy 8 (29%) conservative 10 (37%) unknown	13 (48%) MF	n/a	44	20 (74%)	9 (33.3%)	Median 33 m	n/a
Strobbe et al (1996)	21 (review)	60 (36–75)	21 (100%) salvage mastectomy	n/a	n/a	24 (range,6-82)	16 (76%)	5 (23%)	2Y – 72%, 5Y – 55%	2Y – 35%
Marchal et al (1999)	9	69	9 (100%) Mastectomy	n/a	n/a	16	8 (88%)		Median 15.5m	Median 7.5m
Amajoud et al (2019)	10	73 (64-86)	9 (90%) mastectomy (simple/reconstruction)	n/a	n/a	13 (range, 6-96)	5 (50%)	3 (30%)	2Y – 69%, 5Y – 46%	Median 4 m
Gutkin et al (2020)	35	72 (50-94)	80% mastectomy	11 MF	n/a	43 (range,1.8-203)	12 (34%)	3 (8%)	5Y - 63%	Median 36m
Seinen et al 2012	31	67 (47–89)	24 (77%) – mastectomy 7 (23%) - local excision	n/a	n/a	27 (range 1-151)	19 (61%)	13 (41%)	21/31 DOD	Median 16
Guram et al (2022)	49	74 (56-89)	28 (59%) radical mastectomy 21 (41%) simple/lumpectomy	n/a	n/a	26 (range,4-182)	29 (59%)	3 (6%)	13/49 DOD	3Y RFS 47.8%, 3Y DSS for radical mastectomy 83%.

(P 517) PULMONARY METASTASECTOMY FOR SARCOMA: A SINGLE-INSTITUTION EXPERIENCE

Carlos López Jiménez, MD[1]; Marta Arregui Valles, MD[1]; David Salomón Juliao Caamaño, MD[1]; Ana Gutiérrez, MD[1]; Laura Rodríguez Torres, MD[2]; Antonio Calles Blanco, MD[1]; Carlos Simón Adiego, MDpHD[2]; Guillermo González Casaurrán, MD[2]; Rosa Álvarez Álvarez, PhD, MD[3], (1) Medical Oncology Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain, Madrid, Madrid, Spain, (2) Thoracic Surgery Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain, Madrid, Madrid, Spain, (3) Medical Oncology Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain., Madrid, Madrid, Spain

Objective: Lungs are the most frequent site of metastatic disease for sarcoma patients. Pulmonary metastasectomy (PM) has become a common practice in the setting of sarcoma patients with pulmonary metastatic disease, with an impact on overall survival (OS). Given the heterogeneity, low incidence, and complexity of this group of diseases, its management should be carried out by experienced multidisciplinary teams.
The objective of this study is to review our experience with PM for sarcoma with metastatic involvement of the lungs.

Methods: We conducted a single-institution, observational retrospective study. All sarcoma patients with pulmonary disease who underwent curative intent PM at our center between 1995 and 2022 were included. Clinical and pathological data (age at PM, gender, tumor pathology, primary tumor site), as well as lung disease (distribution, number and size of pulmonary metastases) and treatment features (perioperative chemotherapy, surgical approach, and type of pulmonary resection) were collected from the electronic medical registry. Relapse of disease, repeated PM and outcomes were also registered, and disease-free-interval (DFI) and OS after PM were calculated.

Results: A total of 40 patients were included, 21 (52.5%) female. Mean age was 45.6 years (range 14–77). The most frequent histotypes were leiomiosarcoma (n=12, 30%), synovial sarcoma (n=11, 27.5%), pleomorphic sarcoma (n=4, 10%) and liposarcoma (n=3, 7.5%). Histological grade 3 was present in 21 cases (52.5%). The most frequent primary tumor sites were extremities (n=19, 47.5%), uterus (n= 9, 22.5%) and retroperitoneum (n=5, 12.5%). Primary tumor surgery was performed in 38 (95%) patients, and the median interval between the treatment of primary tumor and PM was 21.5 months (range 2–189).
Metastatic pulmonary involvement was unilateral in 24 patients (60%) and bilateral in 16 (40%) patients. Multiple metastases were detected in 24 patients (60%), while 16 patients (40%) had just a single metastasis. Largest lung metastasis was bigger than 1 cm in 28 patients (68.7%). Thoracotomy was performed as the chosen surgical approach for PM in 36 patients (90%) and thoracoscopy in 4 patients (10%). Wedge was the most frequent type of pulmonary resection (n=30, 75%). 10 patients (25%) received neoadyuvant chemotherapy, and 7 patients (17.5%) received adyuvant chemotherapy.
The median DFI after PM was 8 months (range 1–155), and pulmonary relapse was present in 31 patients (77.5). Repeated PM was performed in 15 patients (37.5%).
The median follow up was 68 months (range 8–345), and the median OS after PM was 44.5 months (range 2–302).

Conclusion: Our study found that PM is a reasonable therapeutic option for metastatic sarcomas with pulmonary disease, with acceptable DFI and OS.

More studies are needed to elucidate which patients are most likely to benefit from this strategy of treatment.

Characteristics of sarcoma patients undergoing PM.

Gender — n (%)	
Male	19 (47.5)
Female	21 (52.5)
Age (years) — mean (range)	45.6 (14–77)
Tumor pathology — n (%)	
Leiomyosarcoma	12 (30)
Synovial sarcoma	11 (27.5)
Pleomorphic sarcoma	4 (10)
Liposarcoma	3 (7.5)
Myxoid liposarcoma	2 (5)
Osteosarcoma	2 (5)
Myxofibrosarcoma	1 (2.5)
Ewing's sarcoma	1 (2.5)
Dermatofibrosarcoma	1 (2.5)
Clear cell sarcoma	1 (2.5)
Chordoma	1 (2.5)
Endometrial stromal sarcoma	1 (2.5)
Histological grade of primary tumor — n (%)	
1	1 (2.5)
2	13 (32.5)
3	21 (52.5)
Missing	5 (12.5)
Primary tumour site — n (%)	
Extremities	19 (47.5)
Uterus	9 (22.5)
Retroperitoneum	5 (12.5)
Pelvis	4 (10)
Thorax	3 (7.5)
Metastatic disease at diagnosis — n (%)	
Yes	3 (7.5)
No	37 (92.5)
Interoperative interval (months) — median (range)	21.5 (2–180)
Distribution of lung metastases — n (%)	
Unilateral	24 (60)
Bilateral	16 (40)
Number of lung metastases — n (%)	
Single metastasis	16 (40)
Multiple metastasis	24 (60)
Surgical approach — n (%)	
Thoracotomy	36 (90)
Thoracoscopy	4 (10)
Type of pulmonary resection — n (%)	
Wedge	30 (75)
Laser	8 (20)
Lobectomy	2 (5)
Resection margin — n (%)	
Negative	38 (95)
Positive	1 (2.5)
Missing	1 (2.5)
Pre-PM chemotherapy — n (%)	
Yes	10 (25)
No	30 (75)
Post-PM chemotherapy — n (%)	
Yes	7 (17.5)
No	33 (82.5)
DFI after PM (months) — median (range)	8 (1–155)
Relapse after PM — n (%)	
Pulmonary relapse	31 (77.5)
Non-pulmonary relapse	7 (17.5)
Non relapse	2 (5)
Repeated PM — n (%)	
Yes	15 (37.5)
No	23 (57.5)
Missing	2 (5)
Outcomes — n (%)	
Death	26 (65)
Alive	10 (25)
Missing	4 (10)
Overall survival after PM (months) — median (range)	44.5 (2–302)

(P 518) OUTCOMES OF NON-ANGIOSARCOMA RADIATION-ASSOCIATED SOFT TISSUE SARCOMAS OF THE CHEST

Leilani Garayua-Cruz, BS[1]; Samuel Broida, MD[2]; Ivy A. Petersen, MD[2]; Scott Okuno, M.D.[2]; Steven Robinson, MBBS[2]; Thanh P. Ho, MD[2]; Brittany Siontis, MD[2]; Matthew T. Houdek, MD[2], (1) University of Puerto Rico School of Medicine, San Juan, Puerto Rico, (2) Mayo Clinic, Rochester, MN

Objective: Radiation-associated soft tissue sarcomas (RA-STS) are a rare complication of patients receiving radiation therapy and are generally associated with a poor prognosis. Most of the literature surrounding RA-STS of the chest is centered on angiosarcoma. Therefore, we aim to document the management and outcome of patients with RA-STS in the chest wall, specifically non-angiosarcoma lesions.

Methods: We conducted a retrospective review of 18 patients from a single institution diagnosed with RA-STS between 1972-2011. RA-STS was defined as a histologically confirmed soft-tissue sarcoma of the chest in a previously irradiated field with a minimum 5-year interval between radiation for the primary tumor and the diagnosis of the sarcoma. All patients underwent surgical resection with curative intent and, for survivors, had at least 12 months follow-up. Exclusion criteria included non-operative treatment and palliative surgical treatment. The outcome measures were local recurrence-free and metastasis-free survival as well as post-operative complications. Perioperative death was defined as intraoperative death or death within 90 days of the index surgery.

The prior malignancies included breast (83%), multiple myeloma (6%), lymphoma (6%), and acute lymphocytic leukemia (6%). All patients underwent radiation treatment for their primary malignancy and dose information was available for 5 patients (mean dose 50 Gy. The median time to secondary malignancy diagnosis was 15 years. The histological diagnoses of RA-STS were undifferentiated pleomorphic sarcoma (72%), fibrosarcoma (11%), malignant peripheral nerve sheath tumor (6%), chondroblastic extraskeletal osteosarcoma (6%), and pleomorphic liposarcoma (6%). Five patients (27%) received chemotherapy, and five patients (28%) received combined treatment with radiation for their metastases. More patients received dual agent chemotherapy (i.e.,etoposide/ifosfomide, gemcitabine/taxotere, ifosfamide/doxorubicin (60%) than with three agents (i.e. methotrexate, doxorubicin, cisplatin; (20%) or four agents (i.e., doxorubicin, gemcitabine, ifosmamide, etoposide; (20%).

Results: All patients were treated surgically. Fourteen patients (78%) underwent wide local excision and four underwent forequarter amputation (22%). Median largest tumor dimensions were 4.8 cm). One patient was positive for 2 foci of tumor at the sternal bony margin, the largest of which measured 8 mm (R2, (6%)). All other patients presented negative margins (R0, n=17 (94%)). Nine patients experienced perioperative surgical complications. Most common reasons for complications were wound-related, including 2 infections and 3 dehiscences. Seven patients (39%) developed local recurrence at a median of 13 months. Three patients (43%) were treated with surgical resection and four (57%) were managed with cryoablation, forequarter amputation, radiation therapy, and chemotherapy with ifosfamide and doxorubicin. While six patients (33%) developed metastases at a median 12.5 months. All six patients acquired metastatic lesions in their lungs, while three of them attained additional lesions in the iliac lymph nodes, right arm, and soft tissue of the neck. Recurrence-free survival at 1 and 5 years was 72% and 50% respectively (Fig. 1). There were no recurrences beyond 19 months from surgery. Disease-specific survival at 1 and 5 years was 89% and 57% respectively (Fig. 2).

Conclusion: Non-angiosarcoma RA-STS involving the chest wall presents a challenging disease process, with surgical management having a high-rate complications. Aggressive surgical management should be considered for patients, as with local control patients can have outcomes similar to patients with Stage IIIB soft-tissue sarcomas.

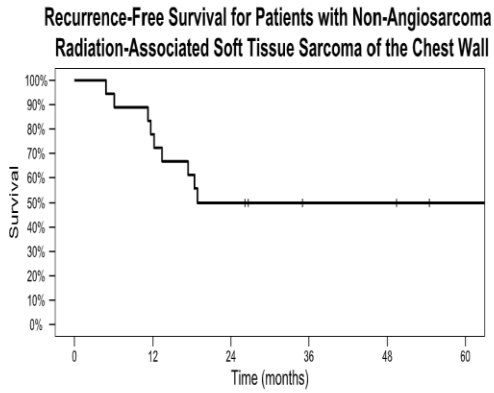


Figure 1. - Kaplan Meier estimate of recurrence-free survival for patients with non-angiosarcoma RA-STS of the chest.

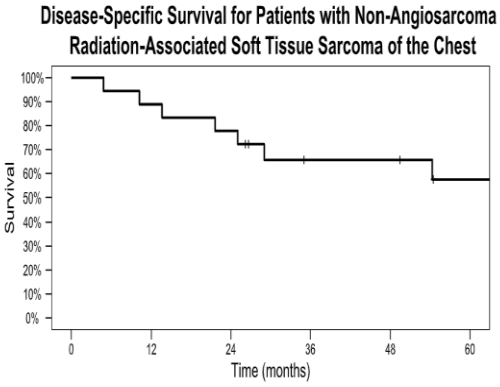


Figure 2. - Kaplan Meier estimate of disease-specific survival for patients with non-angiosarcoma RA-STS of the chest.

(P 519) WHAT ARE THE EXPERIENCES OF GAIT, ACTIVITY LEVELS AND PHYSICAL FUNCTIONING IN PROXIMAL FEMORAL REPLACEMENT AFTER MUSCULOSKELETAL TUMORS?

Ritika Nagarkar, MSc[1]; Sherron Furtado, PhD[2]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[3]; Stephen JG Taylor, MSc PhD[4]; Catherine Pendegrass, n/a[5], (1) University College London, london, England, United Kingdom, (2) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom, (3) Royal National Orthopaedic Hospital, London, England, United Kingdom, (4) University College London, Stanmore, England, United Kingdom, (5) University College London, Greater London, England, United Kingdom

Objective: To evaluate the following in patients who have undergone proximal femoral replacements post musculoskeletal tumors: 1.Impact on physical functioning, quality of life and return to normal living. 2. Relationship between physical functioning, disability and activity levels. 3. Deviations observed in gait and balance, in comparison to healthy control. 4. Rehabilitation interventions useful for this clinical cohort.

Methods: This is an observational and analytical study conducted with the ethical approval obtained from the Royal National Orthopedic Hospital Research and Innovation Centre (Ethics approval: 21/WA/0027). This is a sub-study of STOMP (Stanmore Tumor Outcome Project) which was aimed defining the survivorship experience of patients and the relationship between tumor and patient characteristics and outcomes in patients treated for bone or soft tissue tumors. Patients who met the inclusion criteria were recruited in this study and were selected from hospital wards, clinics or clinical records using convenience sampling. Informed consent from the patients was obtained prior to commencing any assessments, or collecting data. Information about demographics and clinical characteristics from screening of medical notes and investigations were collected. Participants were asked to complete appropriate patient-reported outcomes either in the clinic or at home. This also included wearing a body worn monitor during normal activities in the community (activPAL) which is a small and slim thigh-mounted measurement device that directly measures the postural aspect of sedentary behavior. Via proprietary algorithms, accelerometer-derived information about position and acceleration are used to determine body posture (i.e., sitting/lying and upright) and transition between these postures, stepping, and stepping speed (cadence), from which energy expenditure is inferred indirectly (Edwardson et al., 2017). In addition, existing datasets of information and outcomes collected in routine clinics and as part of hospital records, were analyzed to answer specific objectives e.g., routinely collected Toronto Extremity Salvage Score (TESS) which gives information about functional status of the patient, Quality of Life-Cancer Survivors, Modified Re-integration into Normal Living (mRNL) which measures the degree to which individuals achieve reintegration to normal social activities. Patients were assessed for gait and balance in the Motor Learning Lab (MLL) at RNOH where specific measures of gait were assessed including kinematics of all the joints of the lower extremity, to analyse the deviations observed after the surgical procedure. Kinetic data was also studied, including center of mass velocity and power, which are measures suggestive of deviations in static and dynamic balance.

Results: 13 adult patients (10 males and 3 females) who had undergone proximal femoral replacement for musculoskeletal sarcoma were recruited in this study. They were in the ages ranging from 32 to 93 (Mean age: 66.69±14). The patients recruited in the study underwent surgery between the years 2015 to 2021 (mean years since surgery: 5.14±2.21). Out of 13, 7 patients underwent replacement for the right proximal femur and 6 for the left. In this cohort, the data collection was done for 8 patients in 2022 and for 5 patients in 2023. On an average, data

collection was done 6 years post-surgery for each patient. On analysis the average score for this cohort on the TESS was found to be 58.173±26.2 which indicates a 'fair' score according to the classification, QOL-CS was 53%, and for the mRNL the score was 21.23±8.45. The activity monitoring data obtained from the activPAL indicated total number of steps (mean: 8085), number of sit to stand transitions (mean: 40), activity score (mean: 33.51).

Conclusion: The preliminary findings are suggestive of considerable deviations observed in physical functioning and quality of life. This study is currently in the primary phase and the subsequent analysis is yet to showcase definitive results. This study will focus on the deviations seen in the gait cycle, with respect to kinetics and kinematics, along with impairments seen in specific aspects of physical functioning and its impact of the quality of life as well as normal living of patients.

(P 520) ORTHOPEDIC MANIFESTATIONS OF LYMPHOPROLIFERATIVE NEOPLASMS

Alina Syros, MPH[1]; Meagan Tibbo, MD[2]; Jacob Cohen, MD[2]; Brooke Crawford, MD[2]; Emily Jonczak, MD[3], (1) University of Miami Miller School of Medicine, Miami, FL, (2) University of Miami, Miami, FL, (3) Sylvester Comprehensive Cancer Center / University of Miami Hospital and Clinics, Miami, FL

Objective: Lymphoproliferative disorders are rare inflammatory conditions with the ability to cause musculoskeletal pathology. Rosai-Dorfman, Kimura, and Castleman's disease, seldom present with an orthopedic chief complaint. As a result, there is a paucity of data describing the musculoskeletal manifestations of these lymphoproliferative disorders. This often leads to a delay in diagnosis and treatment. This study serves to better characterize the musculoskeletal manifestations of lymphoproliferative disorders and review management recommendations.

Methods: This is a single-surgeon cohort of 3 patients with unusual lymphoproliferative diagnoses seen at a single tertiary care hospital between March 2022 and March 2023.

Results: Mean age and BMI were 35 years (range 23-51) and 23.7 kg/m2 at the time of diagnosis. All patients were male. On average, 3 biopsies were necessary to reach an accurate diagnosis. One patient required 5 biopsies and was treated with multiple operations and radiation prior to reaching an accurate diagnosis. Alternative diagnoses included sarcoma, lymphoma, granuloma, osteomyelitis, arteriovenous malformation, and nodular fasciitis. All 3 patients required surgery by the senior author for definitive diagnosis and treatment (Table 1 and Figure 1).

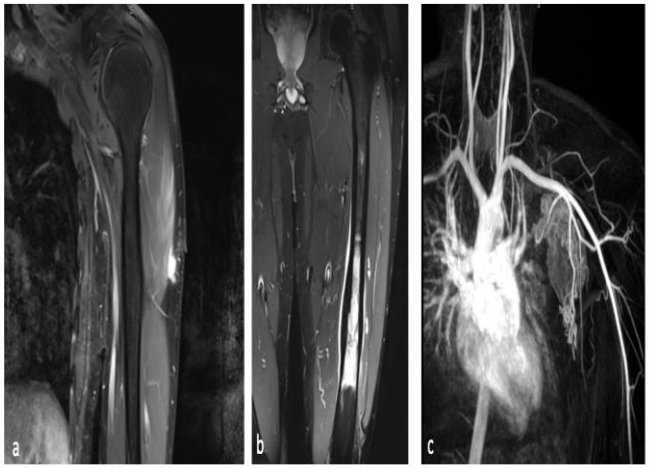
Conclusion: When rare lymphoproliferative disorders occur in the musculoskeletal system, they are difficult to diagnose. History, physical examination, and advanced imaging can be helpful; however, appropriate interpretation of the biopsy by an experienced pathologist is key. This case series highlights 3 of these disorders, common presenting features, differential diagnoses, imaging findings, and treatment.

Table 1: Summary of cases

	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Male
Age (years)	23	51	30
Body Mass Index (kg/m^2)	18.8	28.2	24
Presentation	Asymptomatic deltoid mass	Left thigh pain	Asymptomatic axillary mass
Imaging	Figure 1a	Figure 1b	Figure 1c
Presenting Diagnosis	Sarcoma vs. granuloma vs. nodular fasciitis vs. extraskeletal myxoid fibrosarcoma	Lymphoma vs. osteomyelitis	Arteriovenous malformation vs. sarcoma
Pathology Results	Kimura disease	Rosai-Dorfman disease	Castleman disease
Treatment	No radiation or systemic therapy	Intramedullary reaming 24 Gy XRT* Intravenous vancomycin/cefepime	No radiation or systemic therapy

*XRT: external beam radiotherapy

Figure 1: Presenting images of three cases: a) Coronal T1-weighted fat-suppressed post-contrast MR image of subcutaneous mass in patient 1, b) coronal STIR MR image of femoral involvement in patient 2, and c) coronal MR angiogram depicting numerous aberrant vessels supplying the subpectoral mass in patient 3.



(P 521) HOW TO MANAGE ABDOMINAL PARIETECTOMY, REVIEW OF EXPERIENCE IN INSTITUT BERGONIE

Audrey MICHOT, Plastic surgeon[1]; Magali FAU, Sarcoma surgeon[2]; Sharmini VARATHARAJAH, Sarcoma surgeon[3]; Diane Adam, plastic surgeon[2]; Antoine DANNEPOND, plastic surgeon[2]; Tiphaine MENEZ, plastic surgeon[4]; Grégoire DESOLNEUX, Visceral surgeon[2]; Guillaume BABIN, Pelvic surgeon[2], (1) Institut Bergonié, Bordeaux, Aquitaine, France, (2) Oncological department of Institut Bergonié, Bordeaux, Aquitaine, France, (3) Institut François Baclesse, 14000 CAEN, Basse-Normandie, France, (4) Centre Eugène Marquis, RENNES, Bretagne, France

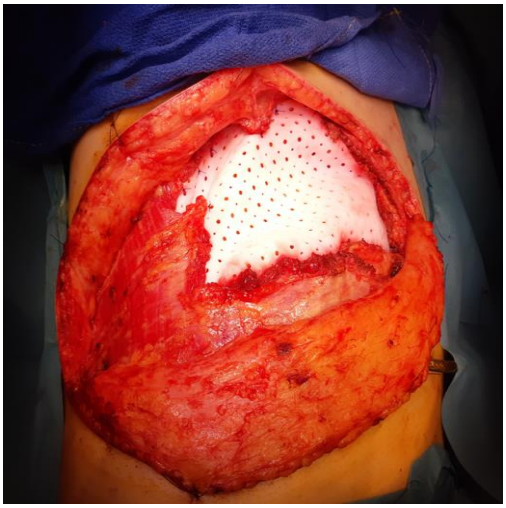
Objective: Conjunctival tumors of the abdominal wall are rare, and usually require extensive surgical excision in conjunction with reconstruction.

Methods: We performed a retrospective analysis including all patients operated on for abdominal wall defect including the inguinal area over the period 2018-2023 at the Institut Bergonié.

Results: Different reconstructive procedures adapted to the defect were proposed and we recorded the postoperative follow-up, any complications and assessed their resumption of sporting activities and the need for girdle support at a distance from this reconstruction.
41 patients were included , 17 for cord sarcoma including 9 gracilis flaps. 12 required muscle excision, 11 with skin defects xx defects combining the 2. 22 flap were used which 9 gracilis, 3 DIEP, 2 ALT and 3 latissimus dorsi. We used two synthetic mesh and six biologic mesh.
We collected data on the rate of complications, re-operations, wound infections and return to sporting and/or professional activities, associated with the need or not to wear a support belt at the last consultation.

Conclusion: We propose a decisional algorithm based on the location of the excision zone and the nature of the defect.







(P 522) FLUORESCENCE GUIDED SURGERY WITH INDOCYANINE GREEN FOR SURGICAL RESECTION OF METASTATIC BONE AND SOFT TISSUE TUMOURS: A CASE SERIES

Sallu Dawo, MBchB[1]; Marcus J. Brookes, MBBS, MRes, MRCS[2]; Corey Chan, MBBS[2]; Kenneth Rankin, FRCS[3], (1) Northern Bone and Soft Tissue Tumour Service, Newcastle, England, United Kingdom, (2) Northern Bone and Soft Tissue Tumour Service, Newcastle upon Tyne, England, United Kingdom, (3) Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, England, United Kingdom

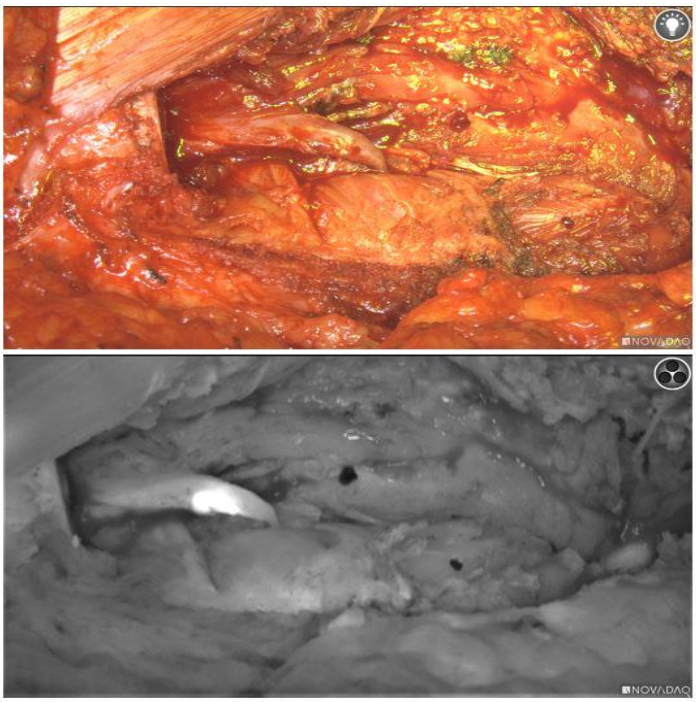
Objective: Bone and soft tissue are common metastatic sites of primary cancers resulting in a considerable workload for orthopaedic oncologists, often requiring operative intervention with the goal usually being the removal of the tumour in its entirety. Intraoperative differentiation between malignant tissue and normal tissue remains a challenge in oncological surgery. Indocyanine green (ICG) is a water-soluble, tricarbocyanine dye with enhanced uptake and

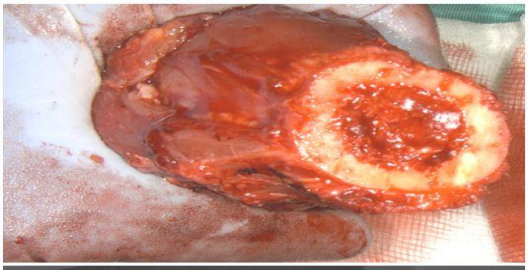
retention in malignant tumour cells which fluoresces in the near-infrared (NIR) spectrum; this can be imaged intra-operatively using handheld NIR cameras. Fluorescence-guided surgery (FGS) with ICG has shown potential as a novel approach in the resection of both lung metastases and solid cancers, including sarcoma in which a reduction in the unexpected positive margin rate has been demonstrated. This study aims to assess the feasibility of FGS using ICG for the resection of metastatic bone and soft tissue tumours.

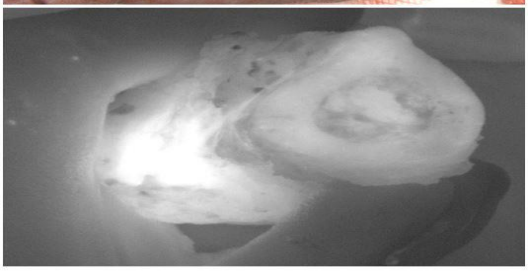
Methods: Patients undergoing resection of bone and soft tissue metastases from any primary tumour type were eligible and a total of 17 patients were recruited. ICG was administered intravenously pre-operatively at a dose of 50-100mg. This was then imaged intra-operatively using the Stryker SPY-PHI camera; any alteration to the operative procedure due to fluorescence was at the discretion of the operating surgeon. Following the procedure, it was recorded whether the tumour had fluoresced and surgeons were asked whether they felt this had guided the procedure. The margin status was assessed by expert pathologists.

Results: Patients with metastatic disease from 12 different primary cancers were enrolled, including lung, breast, renal, colorectal, oesophageal, skin, bone, oropharyngeal and endometrial primaries . All 17 tumours fluoresced intra-operatively, and it was felt to provide intra-operative guidance to the surgeon in 12/17 cases. It was felt to be particularly useful in the identification of residual disease during intralesional surgery and for demarcating the extent of disease during bone resections intraoperatively. Of the 17 patients, 4 surgeries were intralesional procedures and 2 had positive margins due to pathological fractures. Of the remaining 11 patients, 9/11 had negative margins on histopathological assessment. There were no adverse events recorded during this study.

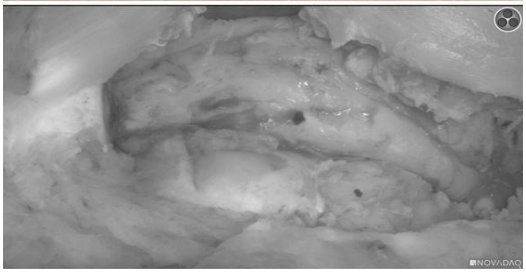
Conclusion: This study demonstrates that FGS using ICG is a promising technology in the management of bone and soft tissue metastases and is feasible across a broad range of cancer types. It is not clear yet however whether this translates into improved patient outcomes and should be investigated further in the form of a prospective randomised control trial.











Reconstruction



Cemented stem with HA collar
On table customisation





Main specimen margins narrowly complete

Wound bed specimen had tumour!

(P 523) IN-BETWEEN SARCOMA: A CATEGORY OF HIGH RISK OF RECURRENCE AND POOR OUTCOME

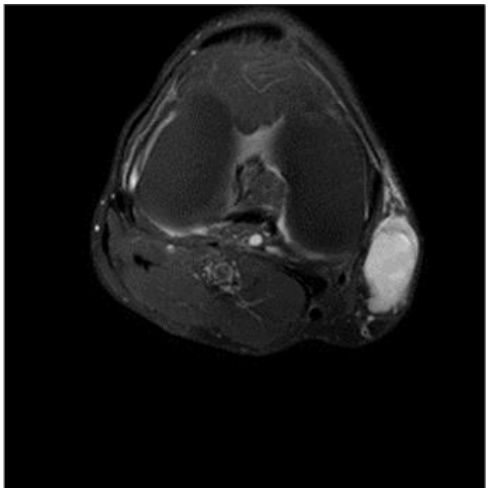
Audrey MICHOT, Plastic surgeon[1]; Diane Adam, plastic surgeon[2]; Eberhard STOECKLE, Sarcoma surgeon[1]; Amandine CROMBE, radiologist[3]; Brice RICHEZ, Anesthesiologist[1]; Maud Toulmonde, N/A, MD[4]; Paul Sargos, Radiation oncologist[1]; Michele KIND, radiologist[1]; Véronique Brouste, Statistician/Data Manager[1]; Antoine Italiano, MD,PhD[1]; Magali FAU, Sarcoma surgeon[2]; François Le Loarer, MD PhD[5], (1) Institut Bergonié, Bordeaux, Aquitaine, France, (2) Oncological department of Institut Bergonié, Bordeaux, Aquitaine, France, (3) CHU Bordeaux, Bordeaux, Aquitaine, France, (4) Institut Bergonie, Bordeaux, Aquitaine, France, (5) Institut Bergnonié, Bordeaux, Aquitaine, France

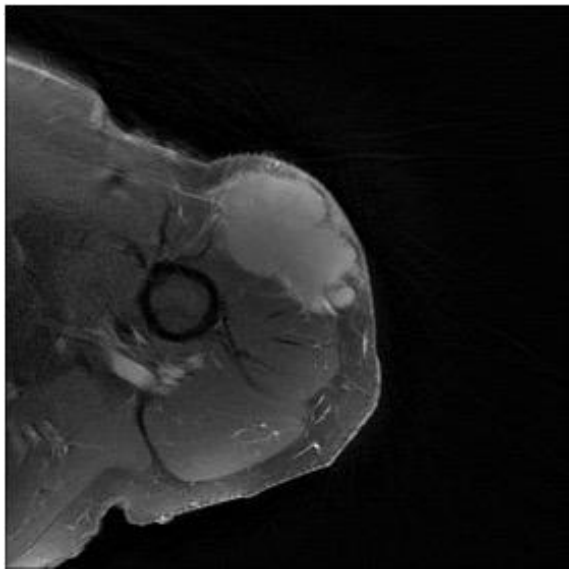
Objective: Tumor depth as a prognostic variable (at either side of the fascia) has been withdrawn from the recent 8th. AJCC classification for soft-tissue sarcoma (STS). However, there are indications that infiltration of the fascia may confer a poor outcome to STS patients.

Methods: We identified limb and trunk wall STS involving the fascia either from inside out (of deep origin) or from outside in (from superficial origin), named In-Between Sarcoma (IBS). We compared retrospectively outcome of IBS patients to those with superficial (sSTS) and deep sarcoma (dSTS).

Results: Of 728 patients, 146 (20%) had IBS, 156 (21%) sSTS and 426 (59%) dSTS. IBS patients presented unfavourable characteristics with larger size (150 mm vs. 40 and 100 mm), more undifferentiated STS (42% vs 26% and 24%), more Grade 3 tumors (59% vs. 29% and 42%), respectively. Postoperative complications were 11.6% vs 3.9% and 8.2% (p = 0.04) and functional impairment 15.7% vs. 4.5% and 15.1% (p = 0.002), respectively.

Conclusion: Overall and metastases-free survival of IBS patients neared survivals of dSTS. Local recurrence-free survival of IBS vs. sSTS and dSTS were 81.1% vs 91.7 and 90.1% (p = 0.01).







(P 524) **PRESENTATION AND OUTCOMES OF EXTRASKELETAL CHONDROSARCOMAS TREATED WITH SURGICAL RESECTION**

Joseph Femino, MD; Lee M. Zuckerman, MD, City of Hope National Medical Center, Duarte, CA

Objective: Chondrosarcomas are malignant tumors arising from cartilage that typically arise in the skeleton. Rarely, these tumors initially present outside of the skeleton and represent only 2% of sarcomas found in the soft tissues. Due to the rarity of these tumors, there is minimal literature regarding their presentation and outcomes. The purpose of this study is to describe the diagnosis, treatment, and prognosis of these sarcomas.

Methods: A retrospective review of all patients who were diagnosed with an extraskeletal

chondrosarcoma at our institution between January 1989 and December 2021 was performed. The patient's age at the time of diagnosis, sex, site and size of the initial tumor, and subtype of chondrosarcoma was collected. In addition to this, treatment modalities, margin status after surgery and surgical complications were obtained in addition to disease free and overall survival.

Results: A total of 11 patients with 12 extraskeletal chondrosarcomas at presentation were identified with 5 males and 6 females. The average age of the patient was 57 (range 14 to 88) and average follow-up was 6.3 years (range 8.1 months to 26.2 years). The size of the tumors ranged from 3.5 cm to 12 cm with the median tumor size being 6.1 cm. Multiple subtypes were diagnosed, including 8 myxoid, 2 mesenchymal, and 1 dedifferentiated extraskeletal chondrosarcomas. Two patients initially presented with lung metastasis. All patients underwent surgical excision, and 6 patients had positive surgical margins and 5 had negative margins. Eight patients received radiation, 7 (64%) patients had a local recurrence, and 6 (55%) patients developed metastatic disease, with 4 (36%) patients who experienced both a local recurrence and developed metastatic disease. All 5 patients that developed metastatic disease had a tumor arise in their lung with one patient who developed a metastatic disease in the inguinal region. Clinical and disease characteristics are reported in Table 1. The overall survival rate was 77% at 3 years and 64% at 5 years. Progression-free survival rate was 70% at 1 year and 50% at 3 and 5 years, respectively. Radiation therapy had no impact on local recurrence (p=1.0). Margin status had no impact on local recurrence (p=0.55) or development of metastatic disease (p=1.0).

Conclusion: Overall, the location of the initial extraskeletal presentation varied, with the majority arising in the appendicular skeleton, and most being myxoid chondrosarcomas. In this series, margin status did not impact local recurrence or the development of metastatic disease. Further studies are necessary to continue to evaluate the presentation and prognosis of these rare tumors.

(P 525) ALL INTERNAL DISTRACTION OSTEOGENESIS AFTER TUMOR RESECTION

Joseph Femino, MD[1]; NADINE Williams, MD[2]; Lee M. Zuckerman, MD[1], (1) City of Hope National Medical Center, Duarte, CA, (2) Loma Linda University, Redlands, CA

Objective: Multiple options for reconstructing bony defects and correcting limb-length discrepancies after tumor resection are available. The use of all internal distraction osteogenesis can be used to reconstruct the defect and correct limb-length discrepancies (LLD's) using the patient's own bone. This study evaluates the outcomes of multiple techniques for all-internal distraction osteogenesis using magnetic lengthening nails.

Methods: A retrospective review of twenty cases of all internal distraction osteogenesis were reviewed. The following data was collected from all patients: age at the time of initial surgery, time to follow-up, location of the surgery, length of distraction osteogenesis performed, number of surgeries performed and complications.

Results: A total of 12 males and 8 females underwent all-internal distraction osteogenesis on 16 femurs, 3 tibiae and 1 humerus. The average age was 32.0 years (range 9-70), the average defect was 10.3 cm (range 3-23) and the average follow-up was 29.9 months (range 3-85). Six patients passed away due to their cancer by final follow-up. Four patients were treated with

lengthening alone for LLD after hemipelvectomy, after an acute shortening or joint replacement. Four patients underwent lengthening after intercalary or osteoarticular allograft reconstruction, five patients underwent plate-assisted bone segment transport (PABST), five patients were treated with a bone transport nail (BTN) and two patients underwent extramedullary distraction osteogenesis. The patients undergoing lengthening for a LLD alone required an average of 1.5 surgeries (range 1-2) and had no complications. The patients undergoing lengthening after allograft reconstruction, only required 1 surgery each with no complications. The PABST patients required an average of 2.6 surgeries (range 1-5). One patient sustained a superficial wound infection requiring surgical debridement. The patients who were treated with a BTN required an average of 4.4 surgeries (range 2-8). Three patients had hardware failure requiring revision surgery, two patients had locking screws back out, one patient had a retained broken intercalary screw that occurred during removal of the nail, and two patients had a non-union. One patient required a revision surgery due to early consolidation of the regenerate and one patient formed poor regenerate requiring grafting. Two of the patients had residual limb-length discrepancies at latest follow-up. For the two cases of extramedullary nailing, each patient required a total of 5.5 surgeries (range 3-8). One patient required 2 revision surgeries due to 2 separate occurrences of hardware failure, and one patient required a revision surgery due to hardware failure.

Conclusion: All-internal distraction osteogenesis after tumor resection provides an option for reconstruction of both intercalary defects and treatment of LLD. Simple lengthening after hemipelvectomy, joint replacement and allograft reconstruction required the least number of procedures and had no complications. When reconstructing an intercalary defect with distraction osteogenesis with or without subsequent lengthening required multiple procedures. The PABST technique had the least number of complications followed by the use of an extramedullary nail and BTN. Although each option provided for limb-salvage with distraction osteogenesis, further research is needed to determine if there is an optimal technique for treatment of these complicated reconstructions.

(P 526) EVALUATION OF THE SURGICAL MANAGEMENT OF THE SKELETON IN PATIENTS WITH OLLIER'S DISEASE AND MAFUCCI SYNDROME

Joseph Femino, MD[1]; Mark Agulnik, MD[2]; NADINE Williams, MD[3]; Lee M. Zuckerman, MD[1], (1) City of Hope National Medical Center, Duarte, CA, (2) City of Hope, Department of Medical Oncology & Therapeutics Research, Duarte, CA, (3) Loma Linda University, Redlands, CA

Objective: Both Ollier’s disease and Mafucci syndrome are rare and have a high rate of skeletal abnormalities. Due to the rare nature of these diseases, the risk of fracture and incidence of surgery performed is not well defined. In addition to surgery for fracture and malignancy, many patients have procedures for deformities and limb-length discrepancies. The purpose of this study is to delineate the number and type of surgical procedures performed in this patient population.

Methods: After obtaining IRB approval, an online survey containing 25 questions was distributed through online support groups to patients with Ollier’s disease and Mafucci syndrome. Both the patient and the immediate family member/caregiver were allowed to respond to the survey.

Results: A total of 123 surveys were obtained from 5 continents including North America (72),

Europe (38), Australia (8), Africa (2) and South America (2). One-hundred nine patients had Ollier's disease and 14 had Mafucci syndrome. The average age of the patient was 27.8 (3-81). Fourteen patients never had surgery on their bones. Seventy-three had at least one fracture which first occurred at 8.6 years of age on average (0.08-29). Eighty-three had surgery due to an enchondroma causing pain or fracture, whereas 14 and 58 patients had surgery to correct a limb-length discrepancy of their arms or legs, respectively. Seventy-nine patient had surgery to correct a deformity. The average age when they first had surgery was 8.9 years (0.5-41) and each patient had an average of 7.4 surgeries (1-49). The most common location for surgery for an enchondroma was the hand, followed by the femur and tibia, and plate fixation was the most common implant. For limb-length discrepancy, the tibia/fibula was the most common surgical site followed by the femur and radius/ulna. Implants included external fixators (42) plates (30) and intramedullary nails (13). Deformity correction was most commonly performed on the tibia/fibula followed by the femur, hand, and forearm with plate fixation (33), external fixation (31) and intramedullary nails (14) as the most common implants. Twenty-four patients developed cancer at an average age of 35.3 (11-50) and 23 were chondrosarcomas. Seven patients developed cancer at a site where a previous surgery was performed and 5 of these patients developed metastatic disease. Five of the initial surgical procedures were to correct a deformity or limb-length discrepancy and 2 of the 3 patients who had intramedullary nails placed developed metastatic disease. The remaining patient who developed metastatic disease had 4 procedures on the same finger with no implant placed.

Conclusion: Surgery is very common in patients with Ollier's disease and Mafucci syndrome, with most patients requiring multiple procedures. The majority of patients have surgery for deformity correction and limb-length discrepancies. Caution should be taken in the skeletally mature patient undergoing these procedures, particularly with an intramedullary nail, to ensure no malignancy is present.

(P 527) SALVAGE OF AN OSTEOARRTICULAR ALLOGRAFT RADIUS RECONSTRUCTION SINGLE BONE TRANSPOSITION WITH WRIST ARTHRODESIS FOLLOWING OSTEOSARCOMA EXCISION

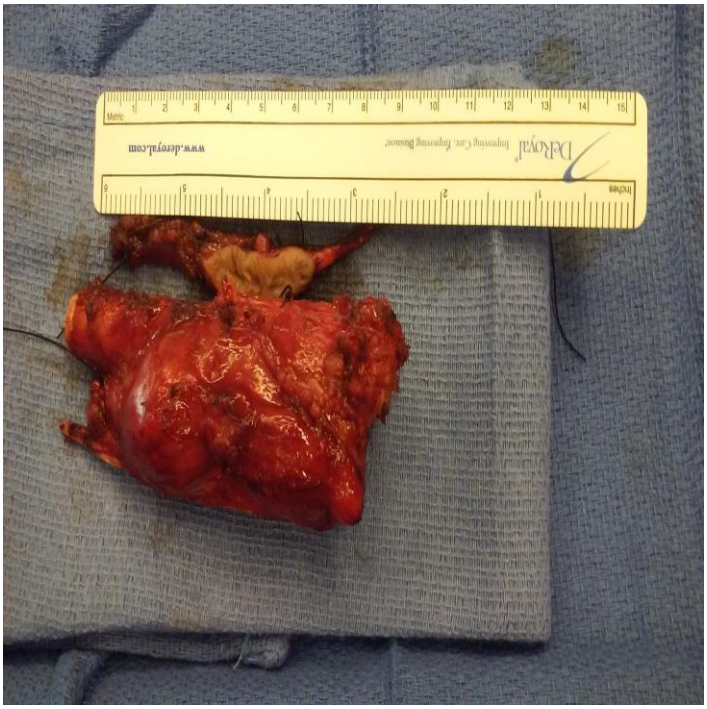
Nadine Williams[1]; Lee M. Zuckerman, MD[2], (1) Loma Linda Medical Cancer Center, Redlands, CA, (2) City of Hope National Medical Center, Duarte, CA

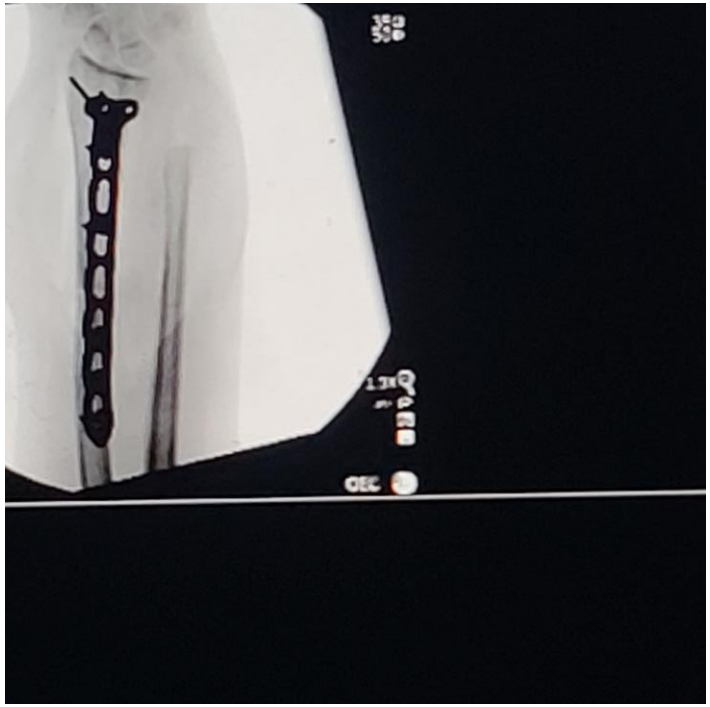
Objective: Reconstruction of the distal radius with an osteoarticular allograft after sarcoma resection can be complicated by both instability and degenerative changes. The purpose of this report is to demonstrate the salvage -progression and treatment of wrist instability and degenerative changes after resection of an osteosarcoma of the radius initially treated with an osteoarticular allograft.

Methods: A 15 year old right hand dominant female had an aggressive high grade conventional osteosarcoma of the left radius. The patient initially underwent resection of 11 cm of distal radius at her initial procedure that was reconstructed with a matched bulk osteoarticular distal radius allograft. Seven months after surgery there was a concern for local recurrence, and 5 cm of the distal ulna was resected. There was no evidence of local recurrence on final pathology. The patient subsequently instability and degenerative changes with pain in her wrist joint and loss of function. Seventeen months after her initial surgery she was treated with arthrodesis of the distal radius allograft to the scaphoid and lunate.

Results: Progressive union at the radial shaft osteotomy site began at approximately 6 months after the initial surgery. The radial shaft osteotomy site demonstrated full fusion 1.5 years post operatively. After fusion of the distal radius osteoarticular allograft to the scaphoid and lunate, the patient had an uneventful post operative course. The patient was able to resume regular fine motor activities with her digits and returned to cheer squad. At her most recent follow- up at 3.5 years after surgery, the patient has 20 degrees of wrist extension, 50 degrees of wrist flexion, near full rotation and a symmetric grip. There is no evidence of local recurrence.

Conclusion: Reconstruction of the distal radius with an osteoarticular allograft to preserve the wrist joint is a useful treatment option for reconstruction of large upper extremity defects post sarcoma resection. Fusion of the allograft can be performed to treat instability and pain due to degenerative changes with maintenance of some wrist range of motion and adequate function.






09/22/2004 STA:ru
MC XR OUTSIDE FILMS ON FILE[BD][AD



Trials in Progress

(P 528) SAFETY AND TOLERABILITY OF ORAL CYCLOPHOSPHAMIDE AND SORAFENIB WITH INTRAVENOUS BEVACIZUMAB WITH THE ADDITION OF ATEZOLIZUMAB IN PEDIATRIC SOLID TUMOR PATIENTS

Jessica A. Gartrell, MD[1]; Anand G. Patel, MD, PhD[2]; Clinton Stewart, PharmD[2]; Elizabeth Stewart, MD[2]; Paul Thomas, PhD[2]; Beth McCarville, MD[2]; Selene Koo, MD, PhD[2]; Teresa Santiago, MD[2]; Benjamin Youngblood, PhD[2]; Caitlin Zebley, MD, PhD[2]; Anthony M. Christensen, PharmD[2], (1) St. Jude Children's Research Hospital, Memphis, TN, (2) St. Jude Children's Research Hospital, Memphis, TN

Objective: There is a growing body of evidence that VEGF plays a pivotal role in creating an immunosuppressive microenvironment and that increased expression of PD-L1 is a mechanism of resistance to anti-angiogenic agents.

This is a Phase 1/2 study evaluating the safety and efficacy of the combination of low dose cyclophosphamide with atezolizumab, bevacizumab, and pharmacokinetically (PK) guided sorafenib in patients with pediatric recurrent, refractory solid tumors. The primary objectives are as follows.

•To establish the safety associated with the administration of the combination of cyclophosphamide, PK-guided sorafenib, bevacizumab and atezolizumab in children and

adolescents/young adults (AYA) with relapsed or refractory solid tumors

•To determine if sorafenib systemic exposure can be successfully targeted to an AUC between 20 and 55 hr·µg/mL by Day 21 of cycle 1 in 60% of evaluable patients, when given in combination with cyclophosphamide, bevacizumab, and atezolizumab in children and AYA with relapsed or refractory solid tumors

•To determine if the combination of cyclophosphamide, PK-guided sorafenib and atezolizumab will result in increased intratumoral T-cell infiltration of CD45RO+ cells between baseline and following two courses of therapy in children and AYA with relapsed or refractory solid tumors following two cycles of therapy

•To characterize the pharmacokinetics of atezolizumab in combination with cyclophosphamide, PK-guided sorafenib and bevacizumab in children and AYA with relapsed or refractory solid tumors

•To assess the feasibility of performing contrast-enhanced ultrasound and explore the correlation between quantitative CEUS parameters and clinical response.

Methods: Patients between the ages of 1-30 years with biopsy-accessible, recurrent or refractory solid tumors with acceptable organ status will be eligible for the study. In addition to standard labs and imaging studies, patients will have a tumor biopsy in conjunction with a contrast-enhanced ultrasound prior to the initiation of study treatment. Treatment will consist of: atezolizumab intravenously, every 3 weeks, Day 1, bevacizumab intravenously, every 3 weeks, Day 1, low-dose cyclophosphamide by mouth once daily, Days 1-21, and sorafenib by mouth every 12 hours, Days 1-21 (Table 1). PK samples of sorafenib will be drawn to achieve a target AUC between 20-55 hr·µg/ml. Each treatment cycle will be 21 days. After completion of two cycles, imaging and the biopsy will be repeated. The ratio of CD45RO+/CD3+ of cells will be calculated, in addition to the total number of CD3+ cells. Treatment will continue if there is at least stable disease and/or an increase in immune cell infiltrate (Figure 1).

Results: .....N/A

Conclusion: .....N/A

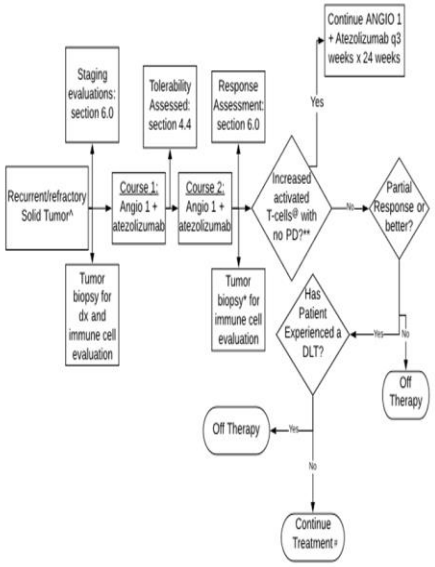


Table 1.

Dose Level	Atezolizumab	Bevacizumab	Sorafenib	Cyclophosphamide
-1C (DLT = myelosuppression) ^	15mg/kg q 21 days	15 mg/kg q 21 days	**exposure level 1	25 mg/m^2 qday
-1B (DLT = HFS or elevation in amylase/lipase)^^	15mg/kg q 21 days	15 mg/kg q 21 days	*exposure level -1	50 mg/m^2 qday
-1A* **	15mg/kg q 21 days	10 mg/kg q 21 days	*exposure level -1	25 mg/m^2 qday
1	15mg/kg q 21 days	15 mg/kg q 21 days	**exposure level 1	50 mg/m^2 qday

**Exposure level -1: AUC between 12 to 33 hr·µg/ml; starting dose 90mg/m^2 q24hrs (see section 5.2 and 6.3)*

***Exposure level 1: AUC between 20 to 55 hr·µg/ml; starting dose 90mg/m^2 q12hrs (see section 5.2 and 6.3)*

**** DLTs are defined in section 4.4. Any DLT with the exception of myelosuppression, HFS, or elevation of amylase/ lipase will require dose reductions as defined in Table 2.*

^ For myelosuppression only cyclophosphamide will be reduced

^^ For HFS or amylase/lipase elevation only sorafenib will be reduced. See Section 4.4.3 for more details.

(P 529) THE MUSCULOSKELETAL TUMOR REGISTRY -- 2023 UPDATE AND OUTLOOK

Adam S. Levin, MD[1]; Benjamin J. Miller, MD[2]; Eric R. Henderson, MD[3]; Nathan W. Mesko, MD[4]; Megan Anderson, MD[5]; George Calvert, MD[6]; Kristy Weber, MD[7]; Joseph Schwab, MD[8]; Meredith Bartelstein, MD[9]; Shalin Shreyaskumar Patel, MD[10], (1) Johns Hopkins University School of Medicine, Baltimore, MD, (2) University of Iowa, Iowa City, IA, (3) Dartmouth/Hitchcock Medical Center, Hanover, NH, (4) Cleveland Clinic, Cleveland, OH, (5) Boston Childrens' Hospital and BIDMC, Boston, MA, (6) Norton Healthcare, Louisville, KY, (7) University of Pennsylvania, Philadelphia, PA, (8) Cedars-Sinai Medical Center, Los Angeles, CA, (9) Memorial Sloan-Kettering Cancer Center, New York, NY, (10) The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: The Musculoskeletal Tumor Registry (MsTR) is a collaboration between the Musculoskeletal Tumor Society (MSTS) and American Academy of Orthopaedic Surgeons (AAOS) to prospectively record oncologic and functional outcomes in patients treated surgically for pelvic and extremity bone and soft tissue sarcoma, as well as metastatic bone disease (MBD). The focus of 2022 was on completing the development of the sarcoma module. At the end of the 2022 calendar year, five institutions are submitting data for collection. Overall, there are currently 28 contracted institutions in various stages of registry framework implementation for expansion of the MsTR.

Methods: The goals for 2023 include: 1) Refinement of the development of the sarcoma modules, 2) Confirm data accuracy and completeness of the sarcoma module inputs, 3) Prioritize grant funding and opportunity for nested clinical trial infrastructure, 4) Continue to increase awareness and enrollment of sarcoma centers, 5) Dissemination of data to sarcoma-relevant organizations. The highest priority is to perform an extensive audit of the collected data in order to ensure integrity related to accuracy, informative, relevance, and parsimony. This is a critical step toward the ultimate goal of reporting reliable expectations and comparisons of oncologic and patient-reported outcomes.

Results: The ideal mechanism for data collection is still being explored throughout the AAOS family of registries, with analytics assessing manually-entered inputs against automated abstracted inputs. To date, all institutions currently submitting have been on the Epic HER platform. Analytical strategies include utilization of Epic for direct assistance, third party contracting, internal analysis through the AAOS Registry Analytics Institute or within a collaborating institution, or providing the option for REDCap entry.

Conclusion: The registry effort continues to progress and 2023 is already yielding significant developments in data auditing, further credentialing, and expansion to interested sarcoma centers. Further efforts are ongoing towards grant proposals and nested clinical trials, publications, and development of REDCap as an alternative data entry platform. Additional development and refinement of smartforms within Epic focus upon minimizing manual data entry while maintaining data integrity.

(P 530) FIH PHASE I STUDY TO EVALUATE THE SAFETY, PHARMACOKINETICS, AND ANTITUMOR ACTIVITY OF THE001 COMBINED WITH REGIONAL HYPERTHERMIA IN PATIENTS WITH LOCALLY ADVANCED OR METASTATIC SOFT TISSUE SARCOMA

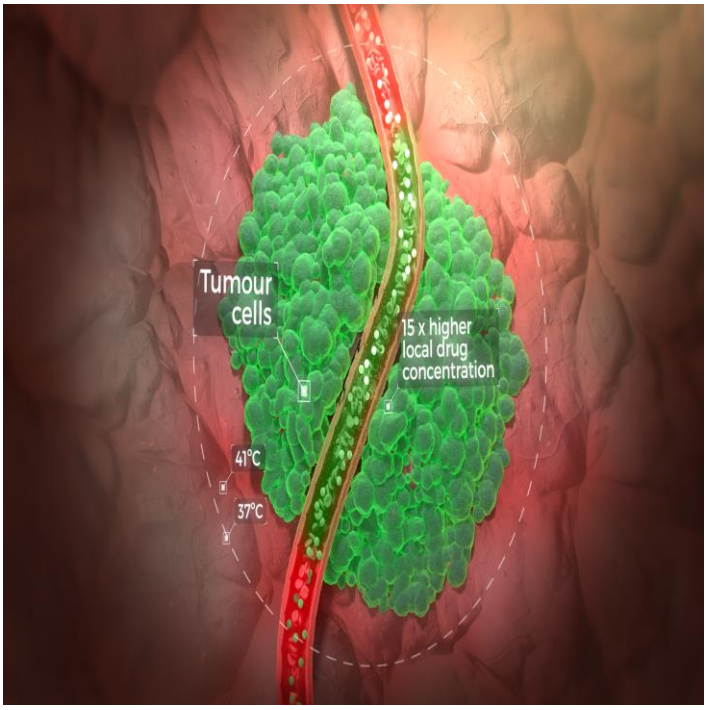
Lars H. Lindner, MD[1]; Dorit Di Gioia, MD[1]; Gesa Schuebbe, MD[1]; Saeed Ghani, MD[2]; Martin Hossann, PhD[3]; Zuzana Haramiova, PhD[3]; Klaus Fischer, PhD[4]; Peter Reichardt, MD, PhD[2], (1) Department of Medicine III, LMU University Hospital, LMU Munich, Munich, Bayern, Germany, (2) Helios Klinikum Berlin-Buch and Medical School Berlin, Berlin, Berlin, Germany, (3) Thermosome GmbH, Martinsried, Bayern, Germany, (4) Thermosome GmbH, Munich, Bayern, Germany

Objective: Doxorubicin (DOX) is the most relevant chemotherapeutic agent for the treatment of soft tissue sarcomas (STS). Because DOX is known to induce immunogenic cell death, combination therapies of DOX with immune checkpoint inhibitors have gained interest. Regional hyperthermia (RHT) has been shown to improve survival in locally advanced high-risk STS when combined with DOX-based chemotherapy, and stimulates the tumor microinvironment to enhance antitumour immune activity. THE001 is a thermosensitive liposomal formulation of DOX based on phosphatidyldiglycerol as the key membrane component. After intravenous administration, THE001 releases DOX in the bloodstream once the temperature in the target area is above 40°C. The application of THE001 plus RHT resulted in preclinical models in up to 15-fold higher DOX concentrations in the tumor and is expected to improve clinical treatment efficacy.

Methods: THE001 in combination with RHT is being evaluated in an open-label, non-randomized, dose-escalation study in patients with locally advanced or metastatic STS (NCT ID: NCT05858710). The study explores ascending doses of intravenous THE001 monotherapy in 21-day cycles to identify the maximum tolerated dose (MTD). From cycle 2, RHT is performed simultaneously. THE001 study began enrolling patients in April 2023 and is conducted at the LMU Klinikum and the Helios Klinikum Berlin Buch. Adverse events are assessed according to CTCAE v5. Tumor response is determined according to RECIST 1.1 and Choi criteria and safety findings reviewed by an independent Data Safety Monitoring Board, which will determine the MTD. Key Phase 1 eligibility criteria include 1) locally advanced (unresectable) or metastatic STS for which treatment with DOX monotherapy is appropriate, and 2) pretreatment with DOX combination chemotherapy (DOX/ifosfamide or DOX/dacarbazine) achieved at least stable disease; for patients who received DOX in an adjuvant setting, local recurrence free interval of >6 months is required, 3) progressive disease not suitable for surgery after 3a) only one further line of chemotherapy (including tyrosine-kinase inhibitor) if the RHT field targets the clinically relevant tumor manifestation/s (e.g., locally advanced or multifocal intraabdominal STS; diffuse metastatic STS in which RHT of a tumor manifestation [e.g., liver] is considered relevant although other systemic metastases are present that do not endanger the patient), or 3b) two or more further lines of chemotherapies (including TKI) for patients with metastatic STS and a tumor manifestation suitable for RHT. Further clinical evaluations of THE001 plus RHT are planned in follow-on studies, e.g. in patients with unresectable localized STS as monotherapy and in combination with ifosfamide (undifferentiated pleomorphic sarcoma) or dacarbazine (leiomyosarcoma) in neoadjuvant therapy of patients with locally-advanced high-risk STS.

Results: Not applicable

Conclusion: Not applicable



(P 531) ENVASARC: A PIVOTAL TRIAL OF ENVAFOLIMAB AND ENVAFOLIMAB IN COMBINATION WITH IPILIMUMAB IN PATIENTS WITH ADVANCED OR METASTATIC UPS OR MFS WHO HAVE PROGRESSED ON PRIOR CHEMOTHERAPY

Richard F. Riedel, MD[1]; Sant P. Chawla, MD, FRACP[2]; Mihaela Druta, MD[3]; Robin L. Jones, BSc, MB BS, MRCP, MD[4]; Scott M. Schuetze, MD, PhD[5]; Joelle Lam, BS[6]; Bonne Adams, MBA[7]; Dongliang Zhuang, PhD[7]; James Freddo, MD[8]; Charles Theuer, MD, PhD[9]; Sandra P. D'Angelo, MD[10], (1) Duke Cancer Institute, Durham, NC, (2) Sarcoma Oncology Center, Santa Monica, CA, (3) H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, (4) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (5) University of Michigan, Ann Arbor, MI, (6) TRACON PHARMECUTICALS, San Diego, CA, (7) TRACON Pharmaceuticals Inc, San Diego, CA, (8) TRACON Pharmaceuticals Inc, Jackson, WY, (9) TRACON, San Diego, CA, (10) Memorial Sloan Kettering Cancer Center, New York, NY

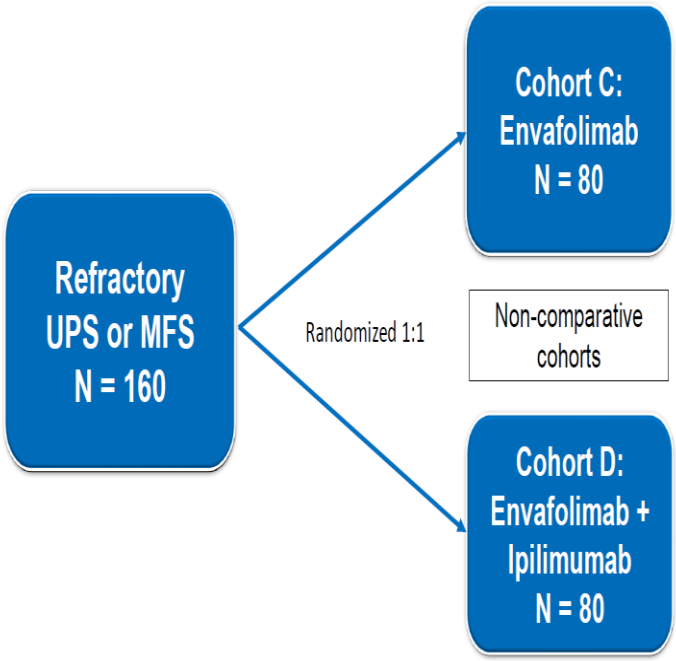
Objective: Metastatic undifferentiated pleomorphic sarcoma (UPS) and the genetically related myxofibrosarcoma (MFS) are soft tissue sarcoma (STS) subtypes with poor prognoses. While responses to first-line chemotherapy can approach 20%, efficacy remains limited in the 2nd-line setting and beyond. Pazopanib, the only FDA approved treatment in the refractory setting, has demonstrated an objective response rate (ORR) of 4%. Envafolimab is a single domain PD-L1 antibody administered by rapid subcutaneous (SQ) injection that is approved for the treatment of microsatellite instability-high (MSI-H) cancer in China. The activity of envafolimab appears to be similar to other PD-1 antibodies administered intravenously (i.v.). Envafolimab demonstrated a 32% ORR in MSI-H colorectal cancer patients with progressive disease following three approved chemotherapeutics, similar to the ORR of 28% and 33% with nivolumab and pembrolizumab in these patients, respectively. The rationale for ENVASARC is based on

envafolimab activity in STS in phase 1 trials and previously reported activity of checkpoint inhibition in UPS/MFS: single-agent pembrolizumab demonstrated a 23% ORR, while the combination of nivolumab and ipilimumab demonstrated a 29% ORR in refractory UPS/MFS. The primary objective of the two parallel cohorts of envafolimab alone and envafolimab in combination with ipilimumab is to demonstrate an ORR with a lower limit of the 95% confidence interval that excludes 5.0% in each cohort. If ≥ 9 responders are observed among the 80 patients enrolled in each cohort, then the lower bound of the 95% confidence interval will exclude 5.0%. Secondary endpoints include duration of response, PFS and OS. Key inclusion criteria: ≤ 2 prior lines of therapy ([neo]adjuvant therapy excluded), ECOG ≤ 1.

Methods: ENVASARC (NCT 04480502) is a pivotal multicenter (at 30 U.S. and U.K. centers) open-label, randomized, non-comparative, parallel cohort study of envafolimab 600 mg every 3 weeks by SQ injection (n=80) or envafolimab 600 mg every 3 weeks by SQ injection combined with ipilimumab 1 mg/kg every 3 weeks i.v. for four doses (n=80) in patients with locally advanced, unresectable or metastatic UPS/MFS with disease progression on one or two lines of prior therapy.

Results: Not applicable

Conclusion: Not applicable



(P 532) MISTOSUS: A PHASE 2 STUDY OF ISCADOR® P IMMUNOTHERAPY FOR PATIENTS WITH RELAPSED OSTEOSARCOMA AND RESECTED PULMONARY METASTASES

Katharine Offer, MD[1]; Nancy Gordon, MD[2]; Marcus Reif, PhD[3]; Douglas J. Harrison, MD[4]; Clark Andersen, MS[5]; Karen Moody, MD[4], (1) Joseph M. Sanzari Children's Hospital, Hackensack Meridian Health (HMH), Hackensack, NJ, (2) The University of Texas MD Anderson Cancer Center, Houston, TX, (3) Iscador AG, Berlin, Berlin, Germany, (4) M.D. Anderson Children's Cancer Hospital (MDACC), Houston, TX, (5) M.D. Anderson Cancer Center, Houston, TX

Objective: The prognosis of pediatric and adolescent/young adult (AYA) patients with relapsed osteosarcoma (OS) is poor, despite complete surgical resection of metastatic disease. Historical studies cite a 12-month event-free survival of 20-35%. Preventing future recurrence of osteosarcoma (OS) in these patients is pivotal to improving survival outcomes. IscadorP (Iscador AG, Arlesheim, Switzerland), is a fermented aqueous extract from naturally derived components of the mistletoe plant (Viscum album L.) grown on pine trees (IscadorP = Pini). In addition to direct cytotoxicity, IscadorP stimulates an immunologic response against tumor cells through interactions with neutrophils, natural killer cells and T-lymphocytes. It is widely used as complementary cancer therapy in Europe. A small study by Longhi et. al. (2014) randomized patients with surgically resected OS metastases to either IscadorP or oral etoposide and showed a 12-month post relapse event free survival (PREFS-12) of 55.5% in the IscadorP group compared to 27.3% in the etoposide group, which persisted at 84-months. The present study, MISTOSUS (NCT05726383), is an open-label, single-arm, phase 2 clinical trial evaluating the efficacy of IscadorP in pediatric/AYA patients with pulmonary relapse of OS. The primary endpoint is PREFS-12 compared to a historical control of 30%. Secondary endpoints include time to relapse and quality of life. Exploratory objectives include correlative studies to evaluate histologic and immunologic response to IscadorP therapy.

Methods: Eligible patients are 8-30 years of age, with at least one episode of OS relapse in the lung. Participants must be within 8 weeks of surgical resection of all gross metastatic disease and have histologic confirmation of relapse. Patients are ineligible if they do not meet organ function requirements, are pregnant, breastfeeding, have another active malignancy, CNS metastases, or have concurrent inflammatory diseases which would confound the results of the study. IscadorP is administered subcutaneously three times weekly (Mon, Wed, Fri) for 13, 4-week cycles. Dosing begins at the lowest dose level and is then titrated up if tolerated and to achieve optimal immune response based on local skin reaction. All patients begin with Series 0 (0.01, 0.01, 0.1, 0.1, 1, 1, 1mg) for minimum of 14 doses, followed by Series 1 (0.1, 0.1, 1, 1, 10, 10, 10mg) for minimum of 14 doses, and then Series 2 (1, 1, 10, 10, 20, 20, 20mg), which is continued through week 52 (total 13 cycles). Patients are removed from study for disease progression or unacceptable toxicity. Target enrollment is 26 patients. PREFS-12 and time to relapse are evaluated based on clinical exams and imaging. Quality of life is assessed with age-based NIH PROMIS® measures. Blood samples from all enrolled patients are analyzed by flow cytometry using T-cell and myeloid functional panels as well as CodePlex Secretome to collect cytokine data. When available, histology of pulmonary tumor specimens occurring after treatment with IscadorP are compared with pre-treatment biopsy/surgery specimens or archival tissue. Flow cytometry and immunofluorescence using multiplex IF Vectra T-cell and myeloid functional panels are also performed on these tumor specimens and compared with baseline archived tissue.

Results: None yet available

Conclusion: An Investigational New Drug application has been obtained through the United States Food and Drug Administration (159616). Institutional Review Board approval has been obtained at Hackensack Meridian Health. This multi-institutional clinical trial will begin enrolling patients in August 2023 at Hackensack Meridian Health in Hackensack, NJ, and MD Anderson Cancer Center in Houston, TX. Clinical trial information: NCT05726383. Results from this study will evaluate the efficacy of IscadorP to improve survival in relapsed lung OS. Funding Acknowledgement: Study funded in part by generous grants from Golfers Against CancerTM and the Susan Zabransky Hughes Memorial Run.

(P 533) SAFETY AND PRELIMINARY BIOLOGICAL ACTIVITY OF A PHASE II TRIAL OF AUTOLOGOUS DENDRITIC CELLS VACCINATION IN SARCOMAS.

Valentina Fausti, MD[1]; jenny Bulgarelli, PhD[2]; Flavia Foca, N/A, n/a[3]; Anna Miserocchi, PhD[4]; Lorena Gurrieri, MD[1]; Nada Riva, MD[5]; francesco De Rosa, MD[6]; Massimo Guidoboni, MD[6]; Massimiliano Petrini, PhD[7]; annamaria Granato, PhD[7]; elena Pancisi, PhD[6]; Nicoletta Ranallo, MD[6]; sebastiano Calpona, N/A, MD[6]; Federica Pieri, MD[8]; roberto Casadei, MD[9]; Emilia Caldari, MD[10]; Fabio Di Cecco, MD[10]; Giorgio Ercolani, MD[11]; Davide Cavaliere, MD[11]; Carlo Alberto Pacilio, MD[11]; Alessandro De Vita, PhD[12]; Silvia Vanni, PhD[13]; Toni Ibrahim, BSc, MSc, MD, PhD[14]; Laura Mercatali, PhD[15]; Laura Ridolfi, MD[16], (1) Clinical and Experimental Oncology, Immunotherapy, Rare Cancers and Biological Resource Center, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", 47014 Meldola, Italy, Meldola, Emilia-Romagna, Italy, (2) IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) "Dino Amadori" S.r.l., Meldola, Emilia-Romagna, Italy, (3) Unit of Biostatistics and Clinical Trials, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Emilia-Romagna, Italy, (4) IRCCS IRST, Meldola, Emilia-Romagna, Italy, (5) Clinical and Experimental Oncology, Immunotherapy, Rare Cancers and Biological Resource Center, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Emilia-Romagna, Italy, (6) Irst, Meldola, Emilia-Romagna, Italy, (7) IRST, Meldola, Emilia-Romagna, Italy, (8) Pathology Unit, "Morgagni-Pierantoni" Hospital, Forlì, Emilia-Romagna, Italy, (9) Orthopedic Unit, Morgagni-Pierantoni Hospital, 47121 Forli, Italy, Forlì, Emilia-Romagna, Italy, (10) Ospedale Morgagni Pierantoni, Forlì, Emilia-Romagna, Italy, (11) General and Oncologic Surgery, "Morgagni-Pierantoni" Hospital, Forlì, Emilia-Romagna, Italy, (12) Istituto Scientifico Romagnolo per lo studio dei Tumori "Dino Amadori", Preclinic and Osteoncology Unit, Meldola, Italy, Meldola, Emilia-Romagna, Italy, (13) Istituto Romagnolo per lo studio dei Tumori "Dino Amadori" IRST IRCCS, Preclinic and Osteoncology Unit, Meldola, Italy, Meldola, Emilia-Romagna, Italy, (14) Istituto Ortopedico Rizzoli IRCCS, Osteoncology- Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, Bologna, Italy, Bologna, Emilia-Romagna, Italy, (15) Istituto Ortopedico Rizzoli IRCCS, Osteoncology- Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, Bologna, Italy ; Istituto Scientifico Romagnolo per lo studio dei Tumori "Dino Amadori", Preclinic and Osteoncology Unit, Meldola, Italy, Bologna, Emilia-Romagna, Italy, (16) IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" , Meldola, Emilia-Romagna, Italy

Objective: Immunotherapy has shown controversial and often disappointing results in patients with soft tissue sarcomas. This is probably due to the great variability and heterogeneity between histologies and to the peculiar immunosuppressive intratumoral infiltrate. In this perspective, the use of an autologous dendritic cell vaccine could be able to reverse this

resistance. The objective of the phase II RaCaD trial is therefore to evaluate the safety and activity of autologous denditic cell vaccination (DCV) after radical metastasectomy in patients affected by rare tumours, including sarcomas.

Methods: Patients affected by soft tissue sarcoma (STS), neuroendocrine tumors, rare head and neck cancers, undergoing radical metastasectomy are included in the study. All Patients receive adjuvant DCV loaded with autologous tumor homogenate followed by IL-2 daily for five consecutive days starting 48 hours after the vaccination by subcutaneous injection. Treatment cycles are repeated every 28±7 days until disease relapse, unacceptable toxicity, completion of the sixth cycle or withdrawal of the consent by the patient, whichever occurs first. A safety analysis was performed after treatment of the sixth patient. In patients who accepted the related biological study (BioRaV), blood samples were collected pre-surgery, before each vaccine administration and at the end of treatment in order to evaluate the vaccine-induced immune response.

Results: Currently, eight patients have been included in the study, five of whom have STS, one patient with myxofibrosarcoma, one with spermatic cord liposarcoma and three with leiomyosarcoma (1 uterine, 1 retroperitoneal, 1 leg). As reported in Table 1, the DCV followed by IL-2 administration is safe with mild toxicities (highest grade toxicities reported: G2 swelling at inoculation site, asthenia, arthralgias). Currently two patients (myxofibrosarcoma patient and retroperitoneal leiomyosarcoma patient) are free from relapse at 10 and 24 months, respectively. Two patients had a local disease recurrence: the patient affected by inguinal liposarcoma after 28 months and the patient affected by limb leiomyosarcoma with pulmonary metastasis after 10 months. One patient showed multifocal metastatic progression after three months of treatment (uterine leiomyosarcoma).
The variations in peripheral blood immune cell populations between DVC cycles are shown in Figure 1. Moreover, to investigate the immunomodulatory activity of the DCV, the following cytokines were evaluated: IL-1b, IL-12, p70, IL-10, IL-2, IFNg, TNF-a, IL-4, IL-6, VEGF, IL-8, Fibronectin. As shown in figure 2, when patients are grouped according to relapse free survival [RFS], patients with a best RFS (group A) compared to those with the worst RFS (group B), some interesting differences can be highlighted:
1) Patients in group A have higher levels and steadily increasing levels of IL-2 compared to group B.
2) Patients in group A have higher baseline values of VEGF which steadily decreases during treatment than group B.

Conclusion: The first safety analysis demonstrated that autologous dendritic cell vaccine is safe and allowed the trial to continue. For the treatment efficacy evaluation more patients need to be included, nevertheless the serum cytokines evaluation showed promising signs of immune-modulating activity. In particular, all patients in which the IL-2 administrated at each cycle was related to a constantly increased IL-2 serum levels showed a better RFS. Moreover, all the patients in which VEGF levels decreased during the treatment had a better RFS; VEGF is well known immune-suppressor and the ability of DCV to reduce VEGF values could be an indication of its activity.

Table 1: Number of patients with reported AEs among patients with at least 1 cycle of treatment

	Grade (%) (n=5 patients)			
	G1	G2	G3	G4
Astenia	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)
Fever	2 (40.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Local reaction at vaccine	5 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Skin, specify[1]	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)
Articular pain	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)

[1] Over swelling at last dose (G2)

Figure 1. Boxplot of peripheral blood count collected during treatment

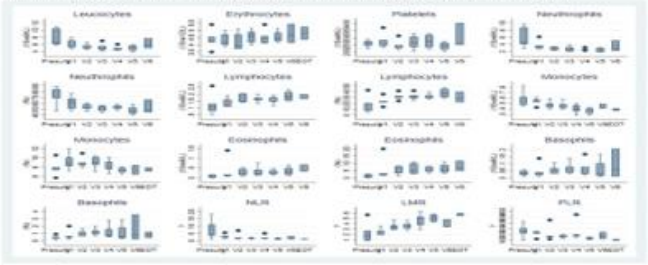
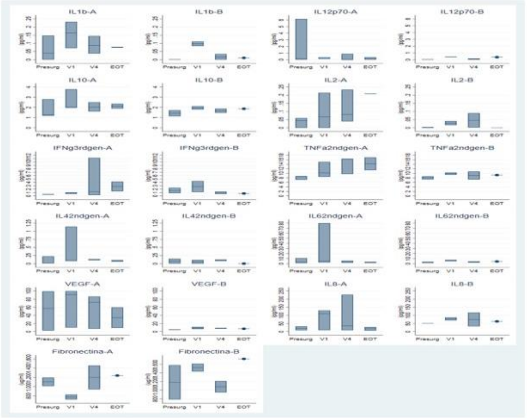


Figure 2. Boxplot of Cytokines collected during treatment.



(P 534) NEOADJUVANT CHEMOTHERAPY COMBINED WITH TARGETED TREATMENT IN HIGH-RISK RETROPERITONEAL SARCOMA – A PROSPECTIVE NON-RANDOMIZED CONTROLLED MULTI-CENTERED STUDY ALL ACROSS CHINA

Jun Chen, MD, PhD[1]; Hanxing Tong, MD, PhD[2]; Xing Wei, MD, PhD[3]; Xuehai xie, MD, PhD[4]; Lantian Tian, MD, PhD[5]; Siwei Tan, MD, PhD[6]; Hongqiang Zhang, MB[7]; Weida Chen, MD, MD[8]; Mei Huang, RN[8]; Shufeng Wang, MD, PhD[9]; Wenjie Li, MD[8]; Yuhong Zhou, MD, PhD[2]; Zhongtao Zhang, MD, PhD[6]; Chenghua Luo, MD, Postgraduate/PhD[10], (1) Retroperitoneal Tumours Centre, Peking University International Hospital, Beijing, China, Beijing, Beijing, China (People's Republic), (2) Shanghai Zhongshan Hospital, Shanghai, Shanghai, China (People's Republic), (3) Department of Medical Oncology, Peking University International Hospital, Beijing, Beijing, China (People's Republic), (4) Peking University First Hospital, Beijing, Beijing, China (People's Republic), (5) The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China (People's Republic), (6) Beijing Friendship Hospital, Beijing, Beijing, China (People's Republic), (7) Fudan University Shanghai Cancer Center Minhang Branch Hospital, Shanghai, Shanghai, China (People's Republic), (8) Department of Retroperitoneal Tumor Surgery, Peking University International Hospital, Beijing, Beijing, China (People's Republic), (9) First Affiliated Hospital Xi'an Jiaotong University, Xian, Shaanxi, China (People's Republic), (10) Retroperitoneal Tumours Centre, Peking University International Hospital, Beijing, China, Beijing, CA

Objective: The role of neoadjuvant therapy in the retroperitoneal sarcoma area remains uncertain. The goal of this clinical trial is to explore the potential survival benefits of neoadjuvant chemotherapy combined with target treatments followed by curative-intent surgery in patients with primary high-risk/grade retroperitoneal sarcoma.

Methods: It is a prospective non-randomized controlled multicentered study. It was approved by Peking University International Hospital Institution Review Board(2022-KY-0070-01) and registered in clinicaltrials.gov(NCT05844813). Patients who meet the inclusion and exclusion criteria will be allocated either into the Surgery-only Group(Group S) or the Neoadjuvant therapy group(Group N) after informed consent is acquired and the pathological confirmation of diagnosis through preoperative biopsy. Patients in Group S will undergo surgeries directly while in the neoadjuvant therapy group, patients will receive neoadjuvant chemotherapy combined with target treatment for three circles followed by the curative-intent surgeries. The drugs in neoadjuvant therapy include pegylated liposomal doxorubicin (PLD), ifosfamide(I) as well as anlotinib(A), a novel multi-targeting tyrosine kinase inhibitor. The sample size is 102 with 51 subjects in each group, calculated through R language. The primary endpoint is 3-year progression-free survival(PFS). Second endpoints include overall survival(OS), local recurrence-free survival(LRFS), and distant recurrence-free survival(DRFS) in 1,3,5 -year durations. The safety and toxicity of neoadjuvant therapy, as well as postoperative morbidities and mortalities, are also collected and evaluated in two groups. Survival outcomes are compared and calculated using the Kaplan-Meier method. Prognostic factors were analyzed through the Cox proportional hazard model. A p-value less than 0.05 was considered statistical significance.

Results: N/A（Not Applicable）

Conclusion: N/A（Not Applicable）

(P 535) THERAGNOSTICS IN SOFT TISSUE SARCOMA USING A VASCULAR DISCRUPTION APPROACH

Antonia Digklia, Dr Med[1]; sarah Boughdad, MD,PHD[2]; Solange Peters, Professor[2]; George Coukos, Professor[3]; John Prior, Professor[2]; Niklaus Schaefer, Professor[2]; Ana Dolcan, MD[2], (1) Department of Oncology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland., Lausanne, Vaud, Switzerland, (2) CHUV, Lausanne, Vaud, Switzerland, (3) Ludwig Institut, Lausanne, Vaud, Switzerland

Objective: Patients with advanced soft tissue sarcomas (STSs) have very poor prognosis and systemic chemotherapy is the mainstay of therapy. Prostate-specific membrane antigen (PSMA) is highly expressed on the vasculature of several solid tumor, including 20% of STS, participating in neovessel growth in developing tumors. We therefore hypothesize that uptake in Ga-68- PSMA-11 PET/CT correlates with PSMA positive tumor neovascularization. Our single center clinical trial aims to evaluate feasibility and efficacy of Lu-177-ITG-PSMA-1 in advanced heavily treated patients with STS. (ClinicalTrials.gov identifier: NCT05420727).

Methods: Patients with STS who progressed after a maximum of two lines of standard treatment with ECOG 0-2 and a life expectancy of >3 months and adequate organ function have been included. The primary objective is the evaluation of tumor growth as judged by the imaging result obtained on Ga-68-PSMA-11 PET/CT scan four weeks after the second cycle of Lu-177-ITG-PSMA-1 treatment, compared to baseline. Secondary endpoints include progression-free survival (PFS), adverse events (AEs), overall survival (OS) and changes in immunological markers. A total of 20 STSs treated at our center will be screened employing PSMA-targeting ligand Ga-68-PSMA-11. Positive patients will be treated with two cycles of Lu-177-ITG-PSMA-1 six weeks apart. A total of 5 patients are expected; accrual is ongoing. Results are expected in 2024.

Results: N/A Trials in Progress.

Conclusion: N/A Trials in Progress.

(P 536) PHASE 1 STUDY OF DISULFIRAM WITH COPPER GLUCONATE AND LIPOSOMAL DOXORUBICIN IN PATIENTS WITH TREATMENT REFRACTORY SARCOMAS

Matteo Trucco, MD[1]; Kurt R. Weiss, MD[2]; Jonathan Mandell, PhD[2], (1) Cleveland Clinic, Cleveland, OH, (2) University of Pittsburgh, Pittsburgh, PA

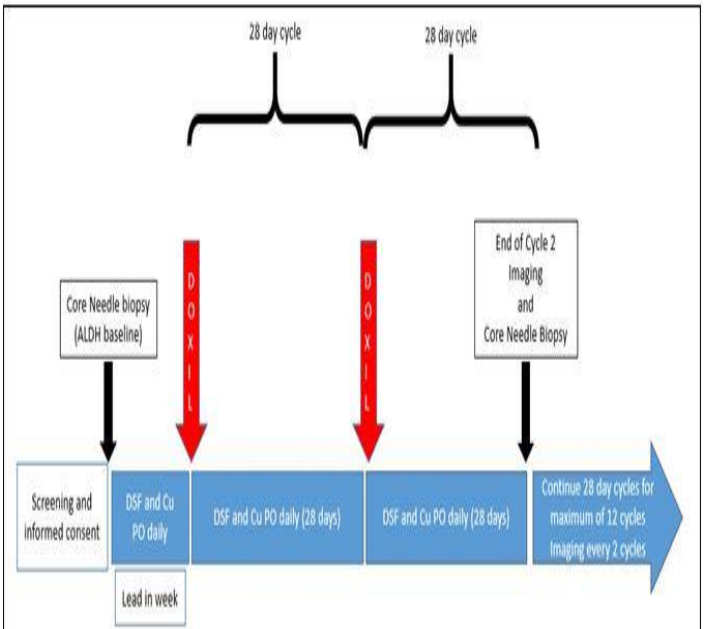
Objective: Relapsed sarcomas carry very poor prognoses and often show resistance to chemotherapy. High expression of the enzyme aldehyde dehydrogenase (ALDH) in sarcomas correlates with aggressive disease and resistance to chemotherapy drugs used to treat these cancers.
Reducing ALDH levels reverses this resistance. Disulfiram (Antabuse) is an inhibitor of ALDH that has been used safely for over 60 years for the treatment of alcoholism. Several pre-clinical studies have demonstrated an anti-cancer effect of disulfiram (DSF), which is enhanced when given with copper (Cu), and its ability to make tumors more sensitive to chemotherapy. We are conducting a clinical trial of disulfiram/copper gluconate (DSF/Cu) in combination with liposomal-doxorubicin in adults with relapsed/refractory sarcomas, with the intention to expand

the study to patients < 18yo once safety is demonstrated in adult patients. Primary objective is to determine the feasibility, safety and tolerability of DSF/Cu in combination with liposomal doxorubicin as measured by adverse events, using CTCAE 5.0 guidelines, in patients with relapsed/refractory sarcomas and establish the recommended phase 2 dose (RP2D) of DSF/Cu in combination with liposomal doxorubicin. Secondary objectives include determining tumor response to the DSF/Cu and liposomal doxorubicin combination using RECIST criteria on imaging, overall and event free survival, and pharmacokinetics of DSF/Cu in combination with liposomal doxorubicin based on samples collected at hour 0, 2, 4 and 24 of Day -6 of lead-in week and Day 1 of cycle 1. Correlative objectives include assessment of ALDH expression and intra-tumoral copper levels in pre- and post treatment tumor biopsies.

Methods: We are conducting a 3+3 dose escalation trial of disulfiram/ copper gluconate with liposomal doxorubicin in patients >18yo with relapsed or refractory sarcomas. Inclusion criteria includes histologically confirmed sarcoma in relapsed or refractory to prior therapy, measurable disease by RECIST criteria, age > 18yo, Karnofskyperformance status ≥50%, adequate organ function, normal serum copper levels and serum cerulopasmin >17mg/dL, able to swallow pills or consume the contents of the DSF and Cu capsules sprinkled on food, ability to understand and the willingness to sign a written informed consent document, abstanance from alcohol during study, agreement to pre- and post-treatment core needle tumor biopsies. Exclusion criteria includes uncontrolled infection, hypersensitivity to any of the agents, patient unwilling or unable to comply with study requirements, known conditions preventing the absorption or safe administration of copper, or Alcohol Use Disorders Identification Test (AUDIT) score of >8 for men and >4 for women. Core needle tumor biopsy will be performed prior to starting therapy and at the end of cycle 2 of treatment.
DSF will be administered orally every morning and Cu administered orally every evening beginning with a lead-in week (Day -6 to 0) and then as part of 28 day cycles. Liposomal doxorubicin will be administered on Day 1 of every 28 day cycle. The dose of Cu will be 5.2mg/m2/day and liposomal doxorubicin will be 30mg/m2 IV throughout the study, while the DSF dose will begin at 225mg/m2/day at dose level 0 and if safe, increased to 300mg/m2/day at dose level 1, or de-escalated to dose level -1 with 150mg/m2/day. Dose-limiting toxicities will be evaluated during cycle 1 while response will be assessed with RECIST criteria after every 2 cycles.

Results: The study opened to enrollment in January 2023 and as of June 2023, 4 patients were enrolled at dose level 0, one patient needing to be replaced per protocol for having to come off study due to progressive disease in the first week of cycle 1 and not having received at least 80% of study drug. Two patients completed 2 cycles of therapy successfully without any dose-limiting toxicities, but had progressive disease at response assessment. The replacement study candidate is soon to begin therapy. Histologies included 1 Ewing sarcoma, 1 osteosarcoma, 1 rhabdomyosarcoma and 1 synovial sarcoma. 3 of the patients had prior doxorubicin exposure. Adverse events at least possibly related to study medication were Grade 2 or lower and included rash, diarrhea, constipation, nausea, vomiting, fatigue, blurred vision, presyncope, and depression.

Conclusion: While the trial is still underway, the combination of disulfiram/copper gluconate with liposomal doxorubicin for the treatment of relapsed or refractory sarcomas appears feasible with no dose-limiting toxicities to date. Blood and tumor samples are being collected for pharmacokinetic and pharmacodynamic analyses. Accrual has thus far been relatively brisk.



Treatment Schema

Dose Level	Liposomal doxorubicin (mg/m2) IV, once every 28 days	Disulfiram (mg/m2/day) PO, daily in AM (rounded for pill size)	Copper Gluconate (mg/m2/day) PO, daily in PM (rounded for pill size)
Level -1	30	150	5.2 (max 9mg/day)
Level 0	30	225	5.2 (max 9mg/day)
Level 1	30	300 (max 480mg/day)	5.2 (max 9mg/day)

(P 537) TGFβ IMPRINTED, EX VIVO EXPANDED UNIVERSAL DONOR NATURAL KILLER CELLS WITH CHEMOTHERAPY FOR RELAPSED BONE AND SOFT TISSUE SARCOMA - NCT05634369, REPORT FROM THE NATIONAL PEDIATRIC CANCER FOUNDATION

Bhuvana Setty, MD[1]; John Mullinax, MD[2]; Masanori Hayashi, MD[3]; Brooke L. Fridley, PhD[2]; Michael Isakoff, MD[4]; Lars M. Wagner, N/A, MD[5]; Jonathan Metts, MD[6]; Matteo Trucco, MD[7]; Damon Reed, MD[2]; Dean Lee, MD, PhD[1], (1) Nationwide Children's Hospital, Columbus, OH, (2) Moffitt Cancer Center, Tampa, FL, (3) Children's Hospital Colorado, Aurora, CO, (4) Connecticut Children's Medical Center, Hartford, CT, (5) Duke Children's Hospital, Durham, NC, (6) Moffitt Cancer Center and Johns Hopkins All Children's Hospital, Tampa, FL, (7) Cleveland Clinic, Cleveland, OH

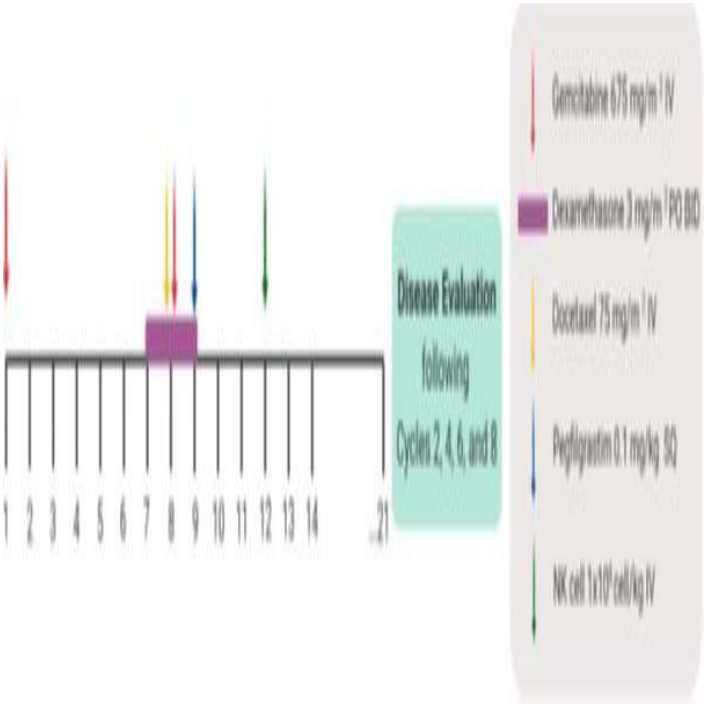
Objective: Background: Bone and soft tissue sarcomas account for 20% of all solid tumor malignancies diagnosed in childhood. The addition of multi-agent chemotherapy improves survival compared to that observed with aggressive surgical local control alone; however, chemotherapy and surgery are not durable for relapsed or primary refractory (R/R) tumors with generally abysmal outcomes (5-year overall survival for R/R osteosarcoma (OS), Ewing sarcoma (ES), rhabdomyosarcoma (RMS), or synovial sarcoma (SS) between 17 and 26%). These poor outcomes highlight the need to develop novel treatment options for patients with relapsed sarcoma. The primary objective of this ongoing trial is to improve the 6-month progression-free survival for children, adolescents and young adults with bone or soft tissue sarcomas.
Rationale: Natural Killer (NK) cells demonstrate innate anti-tumor cytolytic activity in an MHC independent manner. Pre-clinical studies demonstrate that sarcomas are the most NK sensitive cancer type and chemotherapy can increase NKG2D ligand expression that sensitize sarcoma to cytokine activated NK cells. To address the dire need of durable treatment options, a novel cell therapy derived from universal donor (UD) primary NK cells was developed which are expanded utilizing K562 feeder cells expressing 4-1BBL and membrane-bound IL-21 (FC21) for ex vivo growth and activation. A novel non-genetic method of conferring relative TGFβ-resistance to expanded NK cells was induced by the addition of TGFβ during the 14-day expansion process to address the tumor microenvironment suppression of NK cells through TGFβ signaling. These TGFβ-imprinted (TGFβi) NK cells have higher cytotoxicity and are highly resistant to TGFβ suppression. A preparatory regimen of Gemcitabine/Docetaxel will be utilized due to its ability to augment adoptive NK cell immunotherapy by upregulating NKG2D ligands in solid tumors and known activity across relapsed pediatric sarcomas.

Methods: Study design: This clinical trial, NCT05634369, is currently enrolling at one site for initial safety assessment and will open more broadly at National Pediatric Cancer Foundation's 'Sunshine Project' centers over the course of the next year. Patients ≥12 years up to ≤ 40 years of age with relapsed osteosarcoma, Ewing sarcoma, rhabdomyosarcoma, or other soft tissue sarcoma are eligible. Measurable disease is required. Treatment will include up to 8 cycles, 21 days each of chemotherapy preparatory regimen with gemcitabine, docetaxel followed by UD TGFβi ex vivo expanded NK cells. The safety phase of the clinical trial will include 5 patients in each cohort of osteosarcoma, Ewing sarcoma, rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue tumors. After statistical criteria are met for an individual cohort in the safety phase, patients will be enrolled for assessment of efficacy. Tumor responses will be assessed after every two cycles of treatment. Correlative studies include the functional evaluation of NK cells, determination of NK cell sensitization to chemotherapy and minimal residual disease monitoring. For any patients who undergo surgical local control while on study therapy, the impact of the preparatory regimen on tumor sensitization to the NK cells will be assessed. This trial will help further our understanding of how novel cell therapy approaches

can address an unmet need in pediatric solid tumors.

Results: N/A- Trials in Progress

Conclusion: N/A-Trials in Progress



(P 538) IEUROEWING TRIAL FOR LOCALIZED AND METASTATIC EWING SARCOMA

Uta Dirksen, MD[1]; Josephine Kersting, MD[2]; Stefan K. Zöllner, MD[3]; Beate Timmermann, MD[4]; Dimosthenis Andreou, MD[5]; Jendrik Hardes, MD[4]; Arne Streitbürger, MD[6]; Markus Metzler, MD[7]; Eleni Tomazou, Dr.[8]; Dirk Reinhardt, Professor, MD, PhD[9]; Wolfgang Hartmann, N/A, MD[10]; Yasmin Uhlenbruch, MD[11]; Gabriele Calaminus, MD[12]; Andreas Ranft, N/A, Dr.[4], (1) University Hospital Essen, Pediatrics III, West German Cancer Center, German Cancer Research Center (DKTK) site Essen, National Center for Tumordiseases (NCT) site Essen, ,, Essen, Nordrhein-Westfalen, Germany, (2) Pediatrics III, University Hospital Essen, West German Cancer Center, Duesseldorf, Nordrhein-Westfalen, Germany, (3) Pediatrics III, University Hospital Essen, West German Cancer Center, Essen, Nordrhein-Westfalen, Germany, (4) University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (5) Medical University of Graz, Graz, Steiermark, Austria, (6) Sarcomasurgery, Oerthoredic Surgery, University Hospital Essen, West German Cancer Center, Essen, Essen, Nordrhein-Westfalen, Germany, (7) Department of Pediatrics and Adolescent Medicine, University Hospital Erlangen, Erlangen, Bayern, Germany, (8) Children's Cancer Research Institute, St. Anna Kinderkrebsforschung, Vienna, Austria, Vienna, Wien, Austria, (9) GPOH and University Hospital Essen, Essen, Nordrhein-Westfalen, Germany, (10) University Hospital Münster, Münster, Nordrhein-Westfalen, Germany, (11) St. Josef's Hospital Bochum, Bochum,

Nordrhein-Westfalen, Germany, (12) University Hospital Bonn, Bonn, Nordrhein-Westfalen, Germany

Objective: The iEuroEwing trial is a prospective, randomized phase III trial of the European Ewing Sarcoma Group that represents an interdisciplinary treatment protocol jointly developed by European and other international Ewing sarcoma study groups. The primary goal is to improve survival of patients with Ewing sarcoma and related entities (hereafter EWS) by optimizing therapy. iEuroEwing poses three randomized questions in two risk arms, standard risk (SR) and high risk (HR). Systemic neo- and adjuvant polychemotherapy defines as standard intravenous combination therapy of vincristine, doxorubicin, and cyclophosphamide (VDC) alternating with ifosfamide and etoposide (IE).

Methods: The trial consist of two arms: the standard risk arm (SR arm) for patients with localized disease and the high risk arm (HR arm) for patients with primary metastatic disease .The first primary objective is to improve event-free survival (EFS) by introducing maintenance therapy in both the SR and HR B trial arms, randomizing an additive, 6-month maintenance therapy with vinorelbine and cyclophosphamide (VinoCyc). The second primary objective in the SR group is to investigate whether, in the context of local therapy, intensification of the radiotherapy-applied dose in the primary tumor region is non-inferior with respect to the occurrence of toxicity (CTCAE grade ≥ 3) and secondary outcome. The third primary objective is to convert the early response to chemotherapy into a long-term cure for EWS patients in the HR group by allowing additional targeted therapies in independently integrated phase II trials of which two biomarker-driven trials are . Secondary objectives of the iEuroEwing trial are to improve local therapeutic control, event-free survival (EFS), and overall survival (OS) through risk-adjusted and dose-scaled radiotherapy, quality of life and collection of biomaterial and images. The iEuroEwing trial started in December 2022 with a 6.5-year enrollment period and a 2-year follow-up period for a total of 1018 expected patients in 13 Euroean and international countries. The iEuroEwing-led international trial center is the Cooperative Ewing Sarcoma Study (CESS) group. Sponsor of the trial is the Society for Pediatric Oncology and Hematology (GPOH gGmbH). iEuroEwing is funded by German Cancer Aid: DKH70113419 .

Results: N/A trial in progress

Conclusion: N/A trial in progress

(P 539) SARCOMA STEREOTACTIC BODY RADIOTHERAPY AND IMMUNE SYSTEM TRIAL

Erin S. Murphy, MD[1]; Matteo Trucco, MD[1]; Shauna R. Campbell, DO[2]; Jacob G. Scott, MD PhD[3]; Peter M. Anderson, MD, PhD[1]; Stacey Zahler, N/A, MD[1]; Stephanie THomas, MD[1]; Timothy A. Chan, N/A, MD PhD[1]; Dale R. Shepard, MD PhD[1]; George Thomas Budd, MD[1], (1) Cleveland Clinic, Cleveland, OH, (2) Cleveland Clinic Foundation, Cleveland, OH, (3) Cleveland Clinic, Gates mills, OH

Objective: The role of radiotherapy has been limited for patients with sarcoma; however, patients with limited metastases may be curable with aggressive local treatment. Stereotactic body radiotherapy (SBRT) delivers focused high dose radiotherapy in a few treatments, creating a non-invasive locally ablative tool. Early data demonstrate a potential role for SBRT for metastatic sarcoma, but the optimal dose and strategy to incorporate with

systemic/immunotherapy is not yet known. Our preliminary studies show that local control is significantly improved when a biologic effective dose (BED) of ≥ 95 Gy SBRT is used for patients with radiosensitive sarcoma and ≥ 120 Gy for radioresistant sarcoma. We also found that baseline neutrophil to lymphocyte ratio (NTLR) prior to SBRT predicted for overall survival and that change over time correlated with local failure. We hypothesize that utilizing optimal BED of SBRT will result in durable 1-year local control and that NTLR and immune and inflammatory markers over time will predict for local control and survival. Primary Objective: To evaluate the impact of SBRT on the immune system by measuring the neutrophil to lymphocyte ratio for patients with metastatic or unresectable sarcoma. Secondary Objectives To evaluate local control with SBRT for metastatic or unresectable sarcoma using the biologically effective dose (BED) of > 95 Gy of SBRT identified from our previously treated patient population. To estimate rates of acute ≥ grade 3 CTCAE, v. 5.0 adverse events related to SBRT using the biologically effective dose (BED) of > 95 Gy of SBRT. To evaluate the impact of SBRT on the immune system by collecting laboratory data for patients with metastatic or unresectable sarcoma including Immune and inflammatory markers via Olink, Myeloid-derived suppressor cells (MDSC), complete blood count (CBC) with differential, Albumin, Lactate Dehydrogenase (LDH), and C-reactive protein (CRP)

Methods: Patients will receive SBRT to one or more sites using an optimal BED regimen. Systemic therapy will be held for the week of SBRT and the type (more or less lymphocytic) will be documented. Patients will be followed clinically for symptom improvement, control, toxicity, impact on quality of life (via Peds QOL instruments), and survival. Progression will be determined by follow up imaging and/or tumor board consensus. Both local and distant progression will be documented. Local failure will designate as in-field or marginal. In-field recurrences are defined as >80% of the volume of the recurrent lesion was within the 95% isodose line, whereas marginal recurrences are defined as 20% to 80% of the volume of the recurrent lesion within the 95% isodose line. Out of field recurrences are defined as < 20% of the volume of the recurrent lesion within the 95% isodose line. Blood samples will be collected pre- and post-SBRT (within 2 weeks), at follow-up (3 to 6 months), and at 1 year from SBRT. Blood samples include: Immune and inflammatory markers via Olink Panel, Myeloid-derived suppressor cells (MDSC), Complete blood count (CBC) with differential, Albumin, Lactate Dehydrogenase (LDH), and C-reactive protein (CRP). Analytical Methods include phenotypic and functional characterization of immune and non-immune components will involve but not be limited to analysis including flow cytometry, genomics, transcriptomics, metabolomics, spatial phenoptics, and/or assay and/or quantification of soluble components.

Results: NA. Trials in progress submission

Conclusion: NA Trials in progress submission

(P 540) PHASE 2, MULTICENTER, OPEN-LABEL BASKET TRIAL OF NAB-SIROLIMUS FOR MALIGNANT SOLID TUMORS HARBORING PATHOGENIC INACTIVATING ALTERATIONS IN TSC1 AND TSC2 (PRECISION I)

Candace L. Haddox, MD[1]; Erlinda M. Gordon, MD[2]; Gopa Iyer, MD[3]; Lee D. Cranmer, MD, PhD, F.A.C.P.[4]; Kristen N. Ganjoo, MD[5]; Brian Schulte, MD[6]; Li Ding, MS, MA[7]; Anita N. Schmid, PhD[7]; Willis H. Navarro, MD[7]; David J. Kwiatkowski, MD, PhD[8]; Jordi Rodon Ahnert, MD, PhD[9], (1) Dana-Farber Cancer Institute, Boston, MA, (2) Sarcoma Oncology Research Center, Santa Monica, CA, (3) Memorial Sloan Kettering Cancer Center, New York, NY, (4)

University of Washington/Fred Hutchinson Cancer Center, Seattle, WA, (5) Stanford Cancer Center, Palo Alto, CA, (6) UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, (7) Aadi Bioscience, Pacific Palisades, CA, (8) Brigham and Women's Hospital, Boston, MA, (9) MD Anderson Cancer Center, Houston, TX

Objective: nab-Sirolimus, approved in the US for patients with advanced malignant PEComa, is a novel albumin-bound mTOR inhibitor (mTORi) that inhibits the mTOR pathway via suppression of the mTORC1 complex. When TSC1 or TSC2 is inactivated via mutation or loss, the mTOR pathway may be aberrantly activated. TSC1 and TSC2 alterations have been observed in patients with various cancers. Clinically, in an exploratory analysis of the pivotal AMPECT trial of nab-sirolimus in advanced malignant PEComa (NCT02494570), 8/9 (89%) and 1/5 (20%) patients with inactivating alterations in TSC2 and TSC1, respectively, had confirmed response (Wagner, J Clin Oncol, 2021). Most treatment-related adverse events in AMPECT were grade 1/2 (none were grade ≥4) and were consistent with mTORi-class adverse events. In the Expanded Access Program (NCT03817515), patients with various malignancies bearing TSC1 or TSC2 alterations treated with nab-sirolimus showed evidence of response (partial response in 5/7 patients) and manageable toxicities. Based on the clinical observations from AMPECT and the Expanded Access Program, the PRECISION I trial (NCT05103358) was designed to assess the safety and efficacy of nab-sirolimus in a tumor-agnostic study of advanced cancers with TSC1 or TSC2 inactivating alterations.

Methods: Eligible patients are ≥12 years old and mTORi-naïve, possess advanced malignant solid tumors with TSC1 or TSC2 inactivating alterations identified using next-generation sequencing (NGS) in tumor tissue or liquid biopsy (confirmed by central review of NGS reports), and have received appropriate standard treatments, per investigator.

nab-Sirolimus 100 mg/m2 will be given intravenously over 30 min on Days 1 and 8 of each 21-day cycle. The primary endpoint is overall response rate per independent radiographic review (IRR) using RECIST v1.1. Other endpoints include duration of response, time to response, progression-free survival by IRR, overall survival, patient-reported quality of life, and safety.

Enrollment began March 2022. Collaboration with leading NGS providers will expedite the identification of patients with qualifying TSC1 and TSC2 mutations; ongoing study access is facilitated through a just-in-time approach to trial site activation. Based on the prevalence of TSC1 and TSC2 inactivating alterations, the most frequent tumor types expected are bladder, hepatobiliary, endometrial, soft tissue sarcoma, ovarian, and esophagogastric (Table).

Results: Not applicable

Conclusion: Not applicable

Table. Estimated frequency of inactivating *TSC1* or *TSC2* alterations*

Tumor Type	*TSC1* Alterations, %	*TSC2* Alterations, %	*TSC1* or *TSC2* Combined, %
Bladder	6.33	1.70	8.05
Hepatobiliary	1.27	3.31	4.58
Endometrial	2.10	1.22	3.32
Soft tissue sarcoma	1.28	1.71	2.99
Ovarian	1.85	0.92	2.77
Esophagogastric	0.65	1.46	2.11
Non-small cell lung cancer	0.77	1.16	1.93
Colorectal carcinoma	0.99	0.39	1.38
Breast	0.41	0.10	0.51

*The proportion of patients with definite impact alterations (ie, alterations known to have a biological impact, including frameshift, nonsense, and splice-site mutations and deep deletions) derived from analysis of TCGA and cBioPortal by Gulati et al. Data on file. *TSC1*, *TSC2*, tuberous sclerosis complex subunit 1, 2.

(P 541) ETCTN 10563: PHASE 1 STUDY OF PEPOSERTIB AND LIPOSOMAL DOXORUBICIN FOR ADVANCED OR METASTATIC LEIOMYOSARCOMA AND OTHER SARCOMAS

Candace L. Haddox, MD[1]; Karla V. Ballman, Ph.D.[2]; Jan Philipp Novotny, MD, PhD[3]; Geoffrey I. Shapiro, MD, PhD[4]; Steven Gore, MD[5]; Jan Beumer, PharmD, PhD[6]; Adrian Marino-Enriquez, MD, PhD[7]; Jonathan A. Fletcher, MD[8]; Suzanne George, MD[1], (1) Dana-Farber Cancer Institute, Boston, MA, (2) Weil Cornell Graduate School of Medical Sciences, New York, NY, (3) University of Heidelberg, Heidelberg, Baden-Wurttemberg, Germany, (4) Dana-Farber Cancer Institute, Ludwig Center at Harvard Medical School, Boston, MA, (5) National Institutes of Health/National Cancer Institute, Bethesda, MD, (6) University of Pittsburgh Medical Center, Pittsburgh, PA, (7) Brigham and Women's Hospital and Harvard Medical School, Boston, MA, (8) HarvardMedical School/Brigham and Women's Hospital, Boston, MA

Objective: Soft tissue sarcomas (STS) are mesenchymal malignancies comprised of over 80 subtypes with distinct tumor biology and clinical behaviors. STS with complex genomes have chromosomal instability (CIN) that is associated with aggressiveness, but also renders them vulnerable to therapies that cause further genotoxic stress. Leiomyosarcomas (LMS) are among the most common STS and are frequently characterized by CIN and DNA damage response (DDR) dysregulation. Preclinical evidence showed that the gene encoding the catalytic subunit of DNA-PK (PRKDC) is essential in LMS models, suggesting dependency on DNA-PK mediated non-homologous end joining (NHEJ) DNA repair. In preclinical LMS models, low dose anthracycline synergized with the DNA-PK inhibitor peposertib to inhibit tumor growth in vitro and in vivo. We hypothesized that a low, sensitizing dose of liposomal doxorubicin may enhance tumor DNA damage and safely synergize with DNA-PK inhibition in patients with LMS and other STS with CIN and DDR deficits.

Methods: This is a phase 1, open label, multicenter dose escalation and dose expansion study (NCT05711615) evaluating low-dose liposomal doxorubicin given intravenously (IV) on day 1 of 28-day cycles in combination with peposertib given orally twice daily continuously (Table 1). Up to 18 patients over 18 years-old with advanced or unresectable LMS, undifferentiated pleomorphic sarcoma (UPS), myxofibrosarcoma (MFS), dedifferentiated liposarcoma (DDLPS), and synovial sarcoma, ECOG performance status ≤2, who have received at least 1 prior line of systemic therapy (including anthracycline up to 300 mg/m2) are eligible for dose escalation (Figure 1). Dose expansion will be restricted to 12 patients with LMS (Figure 2). The primary objective is to determine the recommended phase 2 dose (RP2D) of the combination based on the DLT rate. The Bayesian Optimal Interval (BOIN) design will inform dose escalation decisions. Secondary endpoints for the expansion cohort include adverse event rate, progression-free survival (PFS), and objective response rate (ORR) per RECIST v1.1. Tumor biopsies during screening and at cycle 1 days 7-10 will be used to evaluate potential predictive biomarkers and assess for changes in DNA damage and repair biomarkers. Circulating tumor DNA (ctDNA) will be collected prior to treatment (dose escalation and dose expansion) and at several timepoints on treatment and at progression (dose expansion only) to correlate ctDNA levels with disease activity and response.

Results: N/A (form would not allow me to submit without including 10 characters minimum)

Conclusion: This ongoing phase 1 dose escalation and dose expansion study is evaluating low dose liposomal doxorubicin combined with the DNA-PK inhibitor peposertib in LMS and select STS with embedded correlative studies to identify predictive biomarkers and explore mechanism of action.

Note: Research support for correlative studies and peposertib supply were provided by the healthcare business of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945).

Dose Escalation Schedule		
Dose Level	Dose	
	Liposomal doxorubicin	Peposertib (tablet)
-1	10 mg/m^2 IV	50 mg PO BID
0*	10 mg/m^2 IV	100 mg PO BID
+1	10 mg/m^2 IV	150 mg PO BID
+2	10 mg/m^2 IV	200 mg PO BID
+3	15 mg/m^2 IV	200 mg PO BID
+4	20 mg/m^2 IV	200 mg PO BID
*Starting dose		

Table 1: Planned dose levels for dose escalation. IV; intravenous; PO, by mouth; BID, twice daily.

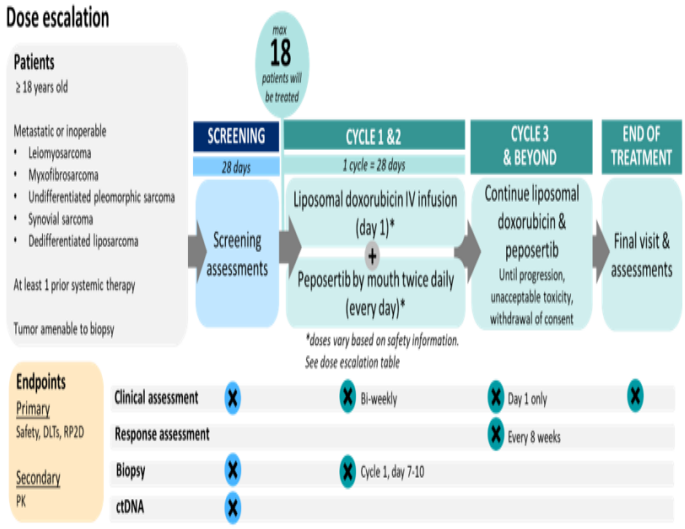


Figure 1: Dose escalation schema. DLTs, dose limiting toxicities; RP2D, recommended phase 2 dose; PK, pharmacokinetics; ctDNA, circulating tumor DNA.

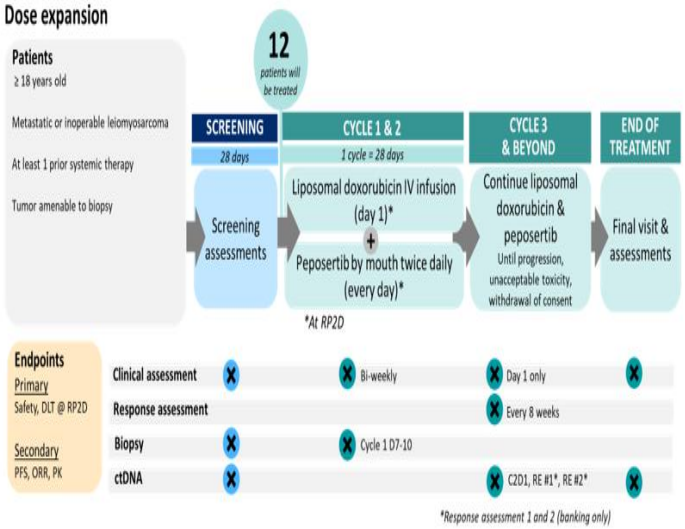


Figure 2: Dose expansion schema. DLT, dose limiting toxicities; RP2D, recommended phase 2 dose; PFS, progression-free survival; ORR, overall response rate; PK, pharmacokinetics; ctDNA, circulating tumor DNA. RE, response assessment.

(P 542) ENROLLMENT OF PEDIATRIC PARTICIPANTS WITH MAGE-A4-POSITIVE SOLID TUMORS IN A PHASE 1/2, OPEN-LABEL, BASKET TRIAL OF AFAMITRESGENE AUTOLEUCEL ("AFAMI-CEL")

Tara O'Donohue, MD[1]; Brian Turpin, DO[2]; Sneha Ramakrishna, MD[3]; Christian M. Capitini, MD[4]; Navin Pinto, MD[5]; Kris Mahadeo, MD[6]; Natalie B. Collins, MD, PhD[7]; Matthew Campbell, MD[8]; Dennis Williams, PharmD[9]; Murray Yule, MD, PhD[10]; Erin Van Winkle, BS[9]; Terri Seiders, RN[9]; Vanessa A. Fabrizio, MD[11]; Amy E. Armstrong, MD[12], (1) Memorial Sloan Kettering Cancer Center, New York, NY, (2) Cincinnati Children's Hospital Medical Center, Cincinnati, OH, (3) Division of Pediatric Hematology, Oncology, Stem Cell Transplantation & Regenerative Medicine, Department of Pediatrics, Stanford University, Palo Alto, CA, (4) Department of Pediatrics and Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, (5) Seattle Children's Hospital, Seattle, WA, (6) Duke University School of Medicine, Druham, NC, (7) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA, (8) UT Southwestern Medical Center, Dallas, TX, (9) Adaptimmune, Philadelphia, PA, (10) Adaptimmune, Abingdon, Oxfordshire, England, United Kingdom, (11) University of Colorado Anschutz, Aurora, CO, (12) Washington University, St. Louis Children's Hospital, St. Louis, MO

Objective: Afami-cel is an affinity-enhanced T-cell receptor (TCR) T-cell therapy genetically engineered to target melanoma-associated antigen A4 (MAGE-A4)-positive solid tumors in human leukocyte antigen (HLA) A*02-eligible patients. In the Phase 2, SPEARHEAD-1 (NCT04044768) trial evaluating afami-cel in participants with advanced/metastatic synovial sarcoma (SS) or myxoid/round cell liposarcoma, encouraging anti-tumor data and an acceptable benefit-to-risk profile are being used to support a Biologics License Application submission. Children and adolescents with rare, MAGE-A4–expressing solid tumors have few treatment options and may also potentially benefit from afami-cel. A Phase 1/2 clinical trial

(NCT05642455) is therefore being initiated to evaluate the safety and anti-tumor activity of afami-cel as well as describe the prevalence of MAGEA4 expression in pediatric patients with relapsed/refractory solid tumors.

Methods: Participants aged 2–21 years old with SS, malignant peripheral nerve sheath tumor, neuroblastoma, or osteosarcoma who express at least one HLA-A*02 inclusion allele and no exclusion allele(s) and whose tumor expresses MAGE-A4 will be eligible for enrollment. Prior to undergoing leukapheresis to collect autologous cells for processing and manufacturing into afami-cel, participants (or their legally authorized representative) will be required to sign a treatment informed consent form. Lymphodepleting chemotherapy consisting of fludarabine (30 mg/m^2/day for 4 days) and cyclophosphamide (600 mg/m^2/day for 3 days) will be administered beginning one week prior to afami-cel infusion. Anti-cancer therapy may be administered between screening and leukapheresis and between leukapheresis and the start of lymphodepletion, but must adhere to mandatory washout periods. Participants will be assigned to one of two groups based on weight and may begin afami-cel dosing in parallel: Group 1 (≥40 kg) will receive a flat dose of 1–10×10^9 transduced T-cells, and Group 2 (≥10 kg to < 40 kg) will receive a weight-based dose of 0.025–0.200x10^9 transduced T-cells per kg. Up to 20 participants will be enrolled. Incidence of dose-limiting toxicities; adverse events (AEs); serious AEs; and the incidence, severity, and duration of AEs of special interest will be assessed along with objective response rate per RECIST v1.1 or the revised International Response Criteria for Neuroblastoma (2017).

Results: N/A. Submitting under Trials in Progress.

Conclusion: N/A. Submitting under Trials in Progress.

(P 543) MANEUVER STUDY: A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTICENTER STUDY OF PIMICOTINIB (ABSK021) TO ASSESS THE EFFICACY AND SAFETY IN PATIENTS WITH TENOSYNOVIAL GIANT CELL TUMOR

Xiaohui Niu, MD[1]; Vinod Ravi, MD, MS, MBA[2]; Tang Liu, M.D.[3]; Yong Zhou, N/A, M.D.[4]; Xiaojing Zhang, M.D.[5]; Zhaoming Ye, N/A, MD[6]; Jingnan Shen, MD[7]; Yi Feng, M.D.[8]; Jing Wang, M.D.[9]; Hongtao Tian, M.D.[10]; Wei Guo, N/A, M.D.[11]; Zheng Tian, M.D.[12]; Yingqi Hua[13]; Tao Li, M.D.[14]; Bingwu Wang, M.D.[15]; Zhichao Tong, M.D.[16]; Huige Hou, M.D.[17]; Xia Zhu, M.D.[18]; Albiruni Ryan Abdul Razak, MB BCh, BM BCh[19]; Ramy Saleh, MD[20]; Michael J Wagner, M.D.[21]; Marian R. Girgis, MD[22]; Juneko Grilley-Olson, MD[23]; Dale R. Shepard, MD PhD[24]; Gabriel Tinoco, MD[25]; Kamalesh K K. Sankhala, M.D.[26]; Hans Gelderblom, MD, PhD[27], (1) Department of Orthopaedic Oncology Surgery, Beijing Ji Shui Tan Hospital, Beijing, Beijing, China (People's Republic), (2) The University of Texas MD Anderson Cancer Center, Houston, TX, (3) The Second Xiangya Hospital, Central South University, Changsha, Hunan, China (People's Republic), (4) Department of Orthopedics, West China Hospital, Sichuan University, Chengdu, Sichuan, China (People's Republic), (5) Department of Bone and Soft Tissue Tumor Surgery, Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Shenyang, Liaoning, China (People's Republic), (6) Department of Orthopedics, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, China (People's Republic), (7) Department of Musculoskeletal Oncology Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China (People's Republic), (8) Department of Orthopaedics, the Second Hospital of Shanxi Medical University, Taiyuan,

Shanxi, China (People's Republic), (9) Department of Sport Surgery and Sport Medicine, Hunan Provincial People's Hospital, Changsha, Hunan, China (People's Republic), (10) Department of Orthopedics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China (People's Republic), (11) Peking University People's Hospital, Beijing, Beijing, China (People's Republic), (12) Department of Orthopaedics, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China (People's Republic), (13) Department of Orthopedic Oncology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, Shanghai, Shanghai, China (People's Republic), (14) Department of Bone and Soft tissue Surgery, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, China (People's Republic), (15) Department of Spine Surgery, Weifang People's Hospital, Weifang, Shandong, China (People's Republic), (16) Department of Osteopathic Oncology, Honghui Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi, China (People's Republic), (17) Department of Bone and Joint Surgery and Sports Medicine Center, The First Affiliated Hospital, Jinan University, Guangzhou, Guangdong, China (People's Republic), (18) Department of Orthopedics, The First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, China (People's Republic), (19) Princess Margaret Cancer Center, Toronto, ON, Canada, (20) MUCH, Montreal, PQ, Canada, (21) Clinical Research Division, Fred Hutchinson Cancer Center, University of Washington, Seattle, WA, (22) Henry Ford Hospital, Henry Ford Cancer Institute, Detroit, MI, (23) Duke University, Durham, NC, (24) Cleveland Clinic, Cleveland, OH, (25) Ohio State University, Columbus, OH, (26) NextGen Oncology, Los Angeles, CA, (27) Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands

Objective: TGCT is a rare type of locally aggressive neoplasm that is primarily caused by overexpression of colony-stimulating factor 1 (CSF1) gene. Pimicotinib is an oral, highly potent, and selective small-molecule antagonist of CSF-1R with minimum inhibition of c-Kit and PDGFR. In a Phase 1b study, Pimicotinib demonstrated significant antitumor activity with the ORR of 77.4% in 50 mg QD cohort by IRC based on RECIST1.1, and a favorable safety profile with no apparent hepatotoxicity (Xu et al, ASCO 2023) in TGCT patients. No apparent ethnic differences were observed for PK and PD data among different ethnic groups based on data from Phase 1 study. Pimicotinib has been granted Breakthrough Therapy Designation (BTD) by China NMPA and US FDA, and PRIority MEdicines (PRIME) Designation by the European Medicines Agency for treating TGCT. MANEUVER study (NCT05804045) is a Phase 3, randomized, double-blind, placebo-controlled clinical trial designed to evaluate the efficacy and safety of Pimicotinib at the dose of 50 mg QD in patients with unresectable TGCT. The study marks the first global Phase 3 trial assessing a CSF-1R inhibitor in TGCT patients across Asia, North America and Europe.

Methods: MANEUVER study consists of two parts. Part 1 is a double-blind phase, approximately 90 eligible patients will be randomized in a 2:1 ratio to 50mg QD ABSK021 treatment group or matching placebo group and will receive consecutive treatment until the completion of Part 1. The randomization will be stratified by geographical location, China vs. non-China sites. All patients who complete Part 1 and meet eligibility criteria will proceed to Part 2. In Part 2, open-label treatment phase, all patients entering this phase will receive open-label 50 mg QD of ABSK021 until the completion of 24 weeks of dosing or withdrawal from the study. Patients who complete 24 weeks of dosing in Part 2 may be eligible for extended treatment. The primary endpoint is 25-week ORR by Blinded Independent Review Committee (BIRC) based on RECIST 1.1. Key secondary endpoints comprise 25-week ORR by BIRC based on Tumor Volume Score, the mean change from baseline in Range of Motion of the affected joint at week 25, the mean change from baseline in the Worst Stiffness and Worst Pain NRS score at week 25, and the mean change from baseline in the PROMIS Physical Functioning score at week 25. Key inclusion criteria include patients that are, histologically

confirmed TGCT and not amenable to surgical resection, existing measurable disease, willingness and ability to complete PRO assessments, symptomatic disease (based on level of pain and stiffness), and age of 18 or older. Key exclusion criteria include previous treatment with highly selective inhibitors targeting CSF-1/CSF-1R (Imatinib and Nilotinib are allowed), known metastatic TGCT or MRI contraindications, major surgery or previous anti-tumor therapy within 4 weeks prior to randomization. An independent Data Monitoring Committee will be established to continuously monitor the safety profile and oversee the overall conduct of the study.

Results: Enrollment is ongoing, approximately 40 sites around the world will participate in this study.

Conclusion: Not applicable

(P 544) INTRAOPERATIVE EVALUATION OF BONE AND SOFT TISSUE SARCOMA SURGICAL MARGINS WITH INDOCYANINE GREEN FLUORESCENCE IMAGING

Matthew F. Gong, MD[1]; William T. Li, MD[2]; Sumail Bhogal, MD[2]; Brittany J. Royes, N/A, MS[1]; Tanya Heim, M.S.[1]; Marcus Malek, MD[3]; Rajeev Dhupar, MD[2]; Stella J. Lee, MD[2]; Richard L. McGough, III, MD[2]; Kurt R. Weiss, MD[1], (1) University of Pittsburgh, Pittsburgh, PA, (2) University of Pittsburgh Medical Center, Pittsburgh, PA, (3) Children's Hospital of Pittsburgh, Pittsburgh, PA

Objective: Achieving negative surgical margins is the cornerstone of bone and soft tissue sarcoma management, with positive margins portending a high risk of local recurrence (LR) and overall poor prognosis. Indocyanine green (ICG) is a fluorophore dye which has been utilized intraoperatively to evaluate surgical margins in several malignancies, including breast and gastrointestinal cancers. Given the critical importance of achieving negative margins in sarcomas, ICG fluorescence imaging has the potential to become a valuable intraoperative margin assessment tool for these uncommon and difficult-to-treat cancers. This study sought to evaluate ICG fluorescence imaging’s utility in bone and soft tissue sarcoma resection.

Methods: This prospective non-randomized clinical trial included patients with biopsy-confirmed bone or soft tissue sarcomas undergoing surgical resection. Exclusion criteria included age < 18 years, prior adverse reaction to iodine or fluorescein, and renal disease. Patients received an intravenous ICG infusion dosed at 2.0 mg/kg three hours preoperatively. Intraoperatively, the removed tumor and tumor bed were imaged using a SPY-PHI fluorescent camera (Stryker Co.; Kalamazoo, MI). ICG-positive margins were defined as regions of tumor bed with a fluorescence intensity ≥77% of the resected tumor’s highest measured intensity (Image 1). Sensitivity and specificity were compared between ICG margins, final pathology, and the surgeon’s blinded clinical impression. Cases where ICG matched or did not match final pathology were then grouped and compared using Student’s t-test and Fisher’s exact test (alpha = 0.05).

Results: N/A Trials In Progress

Conclusion: N/A Trials In Progress

(P 545) NEOADJUVANT PEMBROLIZUMAB AND EFTILAGIMOD ALPHA WITH CONCURRENT RADIOTHERAPY IN PATIENTS WITH SELECTED SOFT TISSUE SARCOMA SUBTYPES (EFTISARC-NEO)

Pawel Sobczuk, MD PhD[1]; Katarzyna Kozak, MD, PhD[2]; Tomasz Switaj, MD, PhD[3]; Aneta Borkowska, MD, PhD[4]; Michał Wągrodzki, n/a[5]; Anna Szumera-Cieckiewicz, MD, PhD[1]; Piotr Rutkowski, MD, PhD[3], (1) Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Mazowieckie, Poland, (2) Maria Sklodowska-Curie National Research Institute of Oncology Warsaw Poland, Warsaw, Mazowieckie, Poland, (3) Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Mazowieckie, Poland, (4) Maria Sklodowska-Curie National Research Institute of Oncology, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw Poland, Warsaw, Mazowieckie, Poland, (5) Department of Pathology and Laboratory Diagnostics, Maria Sklodowska-Curie National Research Institute of Oncology, 02-781 Warsaw, Poland., Warsaw, Mazowieckie, Poland

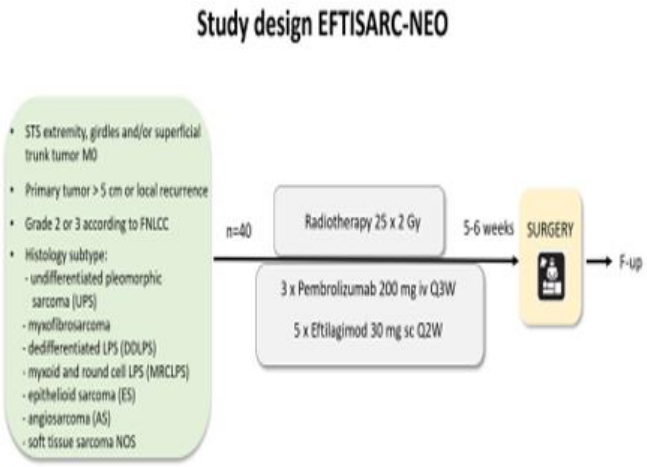
Objective: The efficacy of widely used immune checkpoint inhibitors (anti-PD-1 +/- anti-CTLA4, anti-PD-L1 antibodies) remains modest in patients with advanced soft tissue sarcoma (STS). Only a small proportion of sarcoma patients respond to immunotherapy (ITH), and there are no validated biomarkers of efficacy. To date, the presence of tertiary lymphoid structures appears to be most strongly associated with survival benefits upon treatment with ITH. However, this significantly limits the population of patients who can benefit from ITH. Finding the right combination of immune checkpoint inhibitors remains a major challenge. Therefore, the aim of our study is to evaluate the efficacy and safety of a novel combination of anti-PD-1 antibody and antigen-presenting cell (APC) activator in the neoadjuvant setting. Eftilagimod alpha (Efti) is a dimeric soluble LAG-3 molecule that binds to MHC class II on APCs, thereby inducing T cell proliferation, T helper 1 (Th1) response and NK cell activation (secondary target). Efti is one of the first APC activators that reached phase III clinical trials (advanced breast cancer, head and neck squamous cell carcinoma). Given the low activity of anti-PD-1 monotherapy in STS, we hypothesize that the addition of an APC activator such as Efti may help to activate the cellular immune response mechanism to optimize tumour cell recognition and killing, thus leading to synergistic effects and a higher rate of disease responses. In addition, this dual immunotherapy will be used in combination with radiotherapy (RTH), as preclinical studies have shown that radiotherapy increases the release of tumour-associated antigens, which facilitates cross-presentation of tumour-associated antigens by dendritic cells and priming of antigen-specific T lymphocytes.

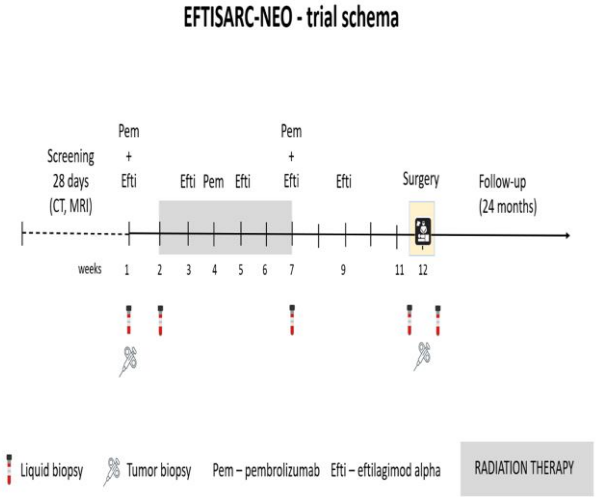
Methods: EFTISARC-NEO is an open-label Phase II study evaluating the efficacy and safety of pembrolizumab and Efti in combination with RTH in the neoadjuvant setting in patients with STS. Key eligibility criteria are: 1) primary or locally recurrent extremity, girdles and/or superficial trunk tumour, 2) one of the following subtypes: undifferentiated pleomorphic sarcoma, myxofibrosarcoma, dedifferentiated liposarcoma, myxoid and round cell liposarcoma, epithelioid sarcoma, angiosarcoma, soft tissue sarcoma NOS; 3) Grade 2/3 tumours according to FNCLCC; 4) Size of the primary tumour>5 cm or locally recurrent of any size. Forty pts will be enrolled to receive efti 30 mg sc Q2W (5 cycles) and pembrolizumab 200 mg iv Q3W (3 cycles) with concurrent RT, followed by surgery. The total radiation dose scheduled is 50 Gy administered in conventional fractionation of 2 Gy/day for 5 days a week from week 2 to week 6. Surgery is planned 5-6 weeks after completion of RTH. The primary endpoint is a percent of tumour hyalinization at the time of surgical resection. Historical data from retrospective analyses indicate that the median fibrosis and hyalinization after preoperative RTH is approximately 15% (H0). With the use of pembrolizumab and eftilagimod alpha, we expect to

increase the rate to 35% (H1). Secondary endpoints include disease-free survival, overall survival, response rate and incidence of adverse events. The exploratory objective of the study is to evaluate local and systemic immunologic responses to neoadjuvant ITH combined with RTH. The correlation between tissue or blood-based biomarkers and clinical outcomes will also be evaluated. Whole-transcriptome sequencing and multiplex immunofluorescence of tumour samples at baseline and after the treatment will be performed to analyse changes in the tumour microenvironment. Additional translational studies based on serial liquid biopsies are planned. Enrolment for the study is ongoing in Poland (EUDRACT number: 2022-003845-36).

Results: Not applicable

Conclusion: Not applicable





(P 546) PRELIMINARY CLINICAL RESULTS OF AN ONGOING PHASE II TRIAL: SPRINT (SARCOMA PREOPERATIVE RADIATION WITH SIMULTANEOUS INTEGRATED BOOST)

Daniela Greto, MD[1]; Mauro Loi, MD[1]; Domenico Andrea A. Campanacci, Full Professor[2]; Federico Sacchetti, MD[2]; Guido Scoccianti, MD[2]; Giuliana Roselli, MD[3]; Annarita Palomba, MD[4]; Marco Banini, MD[5]; Lorenzo Livi, Full Professor[5], (1) Radiation Oncology Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy., Florence, Toscana, Italy, (2) Department of Orthopaedic Oncology and Reconstructive Surgery, Azienda Ospedaliero Universitaria Careggi, Firenze, Italy, Florence, Toscana, Italy, (3) Department of Radiology, Azienda Ospedaliero Universitaria Careggi, Florence, Italy, Florence, Toscana, Italy, (4) Unit of Histopathology and Molecular Diagnostics, Careggi University Hospital, Florence, Italy., Florence, Toscana, Italy, (5) Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy, Florence, Toscana, Italy

Objective: The treatment of localised Soft Tissue Sarcoma (STS) is surgery combined with radiotherapy (RT). Intralesional or Marginal Surgery (R+) are predictive of local recurrence and worse survival. A planned R+ surgery may result from proximity of critical structures to preserve the functionality. In locally advanced STS, preoperative RT is used to downstage the tumor and improve resectability, with an increased risk of wound dehiscence compared to postoperative RT. Modern IMRT may overcome this risk thanks to precise dose modulation that allows selectively spare radiation-sensitive structure (skin) while increasing dose to selected target volumes. SPRINT(Sarcoma Preoperative Radiation with simultaneous INTegrated boost) study was designed to assess the safety and effectiveness of the addition of a preoperative IMRT boost to the potential sites of R+ in STS patients receiving neoadjuvant RT. Here are the early clinical outcomes of the first enrolled patients.

Methods: SPRINT (NCT05761054) is a prospective monocentric single-arm phase II study enrolling locally advanced STS patients eligible for surgery. RT is administered in 25 daily fractions to the MRI-based GTV and peritumoral tissue at risk of microscopic spread (CTV1) to a dose of 50 Gy (2Gy/fraction), with a simultaneous integrated boost to the tumor/dissection plane interface (CTV2) to a dose of 60 Gy (2.4 Gy/fraction). A margin of 0.5 cm is applied to both CTV to obtain PTV1 and PTV2. Concurrent anthracyclines-based chemotherapy is allowed up to 3 cycles. Primary endpoint is the R0 resection rate. Secondary endpoints include pathologic complete response rate, objective response rate, OS, local and distant PFS, acute and chronic toxicity rate. To assess an increase in R0 rate from 81% to 97% assuming β=80% e α=0.05, 33 patients will be included. Dose constraints are shown in Table1. At least 95% of the PTV1 and PTV2 should be covered by 95% the prescription dose up to a maximum allowed dose of 107%.

Results: Five patients were included, with a mean age of 47 years. Tumor was in the limbs and in the trunk in respectively 4 and 1 patient. Concurrent chemotherapy was performed in 2 patients. Epirubicin and ifosfamide-based chemotherapy was administered in both cases. Mean GTV size was 228.6 cm3 (range 59.8-314.5). Mean PTV coverage by the 95% of the dose prescription was 98% (95-100) and 97% (95-100) in PTV1 and PTV2, respectively. Mean PTV1 and PTV2 Dmax were 61.6 Gy (range 59-63) and 64.1 Gy (range 63-66), in both cases below the 107% threshold. Mean Bone Dmax was 55 Gy (56.6-61.8 Gy). Dmax to the skin corridor exceeded 20 Gy in 1 patient (range 12.5-58.1). The NVB was overlapping the PTV2 in all cases, but Dmax was below 66 Gy. All patients underwent surgery with a R0 resection. Two

patients had a pathological complete response. One patient had wound complication requiring VAC therapy.

Conclusion: Planning goals of the first 5 treated patients are achieved in most cases. Preliminary results show a promising tumor response rate.

Table 1. Protocol Dosimetric Goals

Organs at Risk	Planning Objectives
Dmean Bone	<37 Gy
Dmax Bone	<59 Gy
V_{bone} ≥40 Gy	<64%
Dmax Skin Corridor	< 20 Gy
Dmax Neurovascular Bundle	< 66 Gy (EQD2)

D, Dose; V, Volume; GTV, Gross Tumor Volume

(P 547) A PHASE II TRIAL OF TAZEMETOSTAT FOR PATIENTS WITH UNRESECTABLE OR METASTATIC EPITHELIOID SARCOMA (TAZETTA TRIAL)

Shosuke Kita, MD[1]; Tatsunori Shimoi, MD, Ph.D[2]; Keigo Komine, MD, PhD[3]; Masashi Ando, n/a[4]; Kenji Tsuchihashi, MD PhD[5]; Natsuko Okita, M.D., Ph.D.[6]; Ryo Sadachi, n/a[7]; Naoko So, n/a[7]; Satoshi Azuma, n/a[7]; Yoko Kai, n/a[7]; Kan Yonemori, MD, Ph.D[2], (1) National Cancer Center Hospital, tokyo, Tokyo, Japan, (2) Department of Medical Oncology, National Cancer Center Hospital, Tokyo, Japan, Chuo-ku, Tokyo, Japan, (3) Tohoku University Hospital, Sendai, Miyagi, Japan, (4) Aichi Cancer Center, Nagoya, Aichi, Japan, (5) Kyushu University Hospital, Fukuoka, Fukuoka, Japan, (6) Department of Gastrointestinal Medical Oncology, National Cancer Center Hospital, Tokyo, Japan., Tsukiji, Tokyo, Japan, (7) National Cancer Center Hospital, Tokyo, Tokyo, Japan

Objective: Epithelioid sarcoma is an ultra-rare sarcoma that is recognized in less than 1% of patients with soft tissue sarcomas. Patients with surgically unresectable local recurrence or metastatic disease are treated with palliative chemotherapy, but the efficacy of chemotherapy is limited. Approximately 90% of patients with epithelioid sarcoma have SMARCB1/integrase interactor1(INI1) deficiency . The loss of INI1 function leads to overactivation of polycomb repressive complex 2 (PRC2). EZH2 is the catalytic subunit of PRC2, which regulates cell proliferation and is targeted by Tazemetostat, a potent and highly selective EZH2 inhibitor. The clinical efficacy of tazemetostat was evaluated by a multinational Phase 2 open-label basket study comprising 62 epithelioid sarcoma patients (NCT02601950). Tazemetostat has been reported to produce an overall response rate (ORR) of 15% with a median progression free survival (PFS) and overall survival (OS) of 5.5 months (95%CI:3.4-5.9) and 19 months (95% CI:11- not estimable) respectively. Here we present a phase 2 trial (TAZETTA trial; NCCH2107 trial) that will evaluate the efficacy and safety of tazemetostat in patients with advanced epithelioid sarcoma in Japan as a sub-study of the nationwide large registry project for rare cancers in Japan (MASTER KEY Project).

Methods: TAZETTA study is a non-randomized, single-arm, phase 2 study of tazemetostat in epithelioid sarcoma. Eligible patients with locally advanced or metastatic epithelioid sarcoma with loss of INI1 who have received previous treatment, including doxorubicin, an ECOG PS of 0 or 1 or 2, and ≥1 measurable lesion by RECIST v1.1. Tazemetostat will be administered 800 mg twice daily as a single oral agent in 28-day cycles. The primary endpoint is progression free survival rate at 18 weeks. The secondary endpoints include PFS, OS, ORR, disease control

rate, duration of response and safety. Biomarker samples will also be collected for analysis.Ipsen provided a courtesy review of this presentation, the review was for scientific accuracy only, and no input on content was provided.

Results: Not Applicable

Conclusion: Not Applicable

(P 548) A PHASE 2/3 STUDY EVALUATING THE EFFICACY AND SAFETY OF UNESBULIN IN ADVANCED LEIOMYOSARCOMA (SUNRISELMS)

Sant P. Chawla, MD, FRACP[1]; Rashmi Chugh, MD[2]; Jean-Yves Blay, MD, PhD[3]; Robin L. Jones, BSc, MB BS, MRCP, MD[4]; John D. Baird, PhD[5]; Dhiren D'Silva, MBA[5]; Kylie O'Keefe[5]; Mark Rance, MBBS, MRCS[5]; Marla Weetall, PhD[5], (1) Sarcoma Oncology Center, Santa Monica, CA, (2) University of Michigan Rogel Cancer Center, Ann Arbor, MI, (3) Centre Léon-Bérard, Lyon, Rhone-Alpes, France, (4) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (5) PTC Therapeutics, Inc., South Plainfield, NJ

Objective: Leiomyosarcoma (LMS) is one of the most common and aggressive adult soft tissue sarcoma subtypes, with a high risk of recurrence and decreased disease-specific survival (George S, et al. J Clin Oncol. 2018; 36:144–150). A high unmet need remains for treatment of patients with unresectable or metastatic, relapsed or refractory LMS. In a Phase 1b study (NCT03761095), unesbulin, a microtubule polymerization inhibitor, plus dacarbazine (DTIC), was evaluated in patients with unresectable or metastatic, relapsed or refractory LMS, with acceptable benefit/risk ratio to support the start of a Phase 2/3 study. Here, we report the study design of the Phase 2/3 study SUNRISELMS that will evaluate the efficacy and safety of the combination of unesbulin with DTIC in patients with advanced LMS who have received at least one prior line of systemic therapy (NCT05269355).

Methods: SUNRISELMS is an international, randomized, double-blind, placebo-controlled, Phase 2/3 study comparing the efficacy and safety of unesbulin + DTIC versus placebo + DTIC in patients with unresectable or metastatic, relapsed or refractory LMS who received ≥ 1 prior line of systemic therapy. Patients will be randomized 2:1 to unesbulin 300 mg orally twice weekly (BIW) in each 3-week treatment cycle plus DTIC 1000 mg/m2 intravenous (IV) once every 21 days (Q21D). Matching oral placebo BIW plus DTIC IV 1000 mg/m2 will be administered Q21D. An interim analysis will be performed when ~36%, or 88 of the progression-free survival (PFS) events occur. Eligible patients will have unresectable or metastatic, relapsed or refractory, measurable LMS according to RECIST 1.1, and be ≥18 years. Approximately 345 patients will be enrolled: 300 patients with 1 to 3 prior lines of systemic therapy for the primary analysis and 45 patients with at least 4 prior lines of systemic therapy for the exploratory interim analysis. Randomization will be stratified by histological tumor type (uterine versus nonuterine LMS), ECOG score of 0 or 1 and lines of treatment. The primary endpoint is PFS. Key secondary endpoints include overall survival, objective response rate, disease control rate, duration of response, and safety. The study is open and recruiting patients (NCT05269355).

Results: This section does not apply to this abstract since it is a trial in progress.

Conclusion: This section does not apply to this abstract since it is a trial in progress.

(P 549) DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL OF AL102 FOR TREATMENT OF PROGRESSING DESMOID TUMORS: THE RINGSIDE PHASE 3 STUDY DESIGN

Piotr Rutkowski, MD, PhD[1]; Mrinal Gounder, MD[2]; Robin L. Jones, BSc, MB BS, MRCP, MD[3]; Jonathan Y. Yovell, MD[4]; Gary Gordon, MD, PhD[5]; Andres A. Gutierrez, MD, PhD[6]; Bernd Kasper, MD, PhD[7], (1) Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Mazowieckie, Poland, (2) Memorial Sloan Kettering Cancer Center, New York, NY, (3) Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, England, United Kingdom, (4) Ayala Pharmaceuticals, Rehovot, Tel Aviv, Israel, (5) Ayala Pharmaceuticals, Northbrook, IL, (6) Ayala Pharmaceuticals, Monmouth Junction, NJ, (7) University of Heidelberg, Mannheim University Medical Center, Mannheim, Baden-Wurttemberg, Germany

Objective: There is an unmet need for effective systemic therapy for desmoid tumors (DT, aggressive fibromatosis) that provides durable tolerability, symptom improvement, and tumor regression. Gamma secretase inhibitors (GSIs) demonstrate antitumor activity against DT. AL102 is a potent, orally available, selective GSI under investigation as an antineoplastic agent.

Methods: RINGSIDE (AL-DES-01) is a 2-part Phase 2/3 study. In the open-label Phase 2 study (Part A), adults with progressing DT (≥10% unidimensional growth within 18 months or DT-related pain requiring non-opioid medication) were randomized to three dosing regimens: 1.2 mg once daily, 2 mg intermittent BIW (2 days on 5 days off), or 4 mg intermittent BIW. RINGSIDE Phase 3 (Part B) is a double-blind, placebo-controlled study evaluating the chosen dose regimen from Phase 2 (1.2 mg once daily) in adults and adolescents (≥12 years of age) with recurrent or treatment-naïve histologically confirmed progressing DT per investigator. For eligibility, progression is defined as ≥20% measured by MRI or CT scan according to RECIST v1.1 within 12 months of the screening visit. Planned enrollment is for ≈156 subjects globally to be randomized 1:1 to either AL102 1.2 mg once daily or placebo. Randomization will be stratified according to tumor location: intra-abdominal vs. extra-abdominal (including abdominal wall). Subjects will undergo MRI or CT scans (using the same modality throughout the study) every 12 weeks to assess tumor response according to RECIST v1.1 by blinded independent central review (BICR). The primary endpoint is progression-free survival (PFS) by BICR based on RECIST v1.1. Primary and secondary endpoints are summarized in Table 1. The trial is currently enrolling.

Results: Not applicable

Conclusion: Not applicable

Table 1. RINGSIDE Phase 3 Study Endpoints

Objectives	Endpoints
Primary	
Evaluate effects of AL102 on disease progression	• PFS defined as time from randomization until date of assessment of progression (assessed by BICR based on RECIST v1.1) or death by any cause
Secondary	
Evaluate additional effects of AL102 on tumor response	• ORR (CR and PR) by BICR based on RECIST v1.1 • DOR defined by time from CR or PR (by BICR based on RECIST v1.1) until first documentation of disease progression or death from any cause
Evaluate effects of AL102 on quality of life	• Change from baseline in quality of life as determined by: – GOunder/Desmoid Tumor Research Foundation (DTRF) DEsmoid Symptom Scale and Impact Scale (GODDESS) – Patient-Reported Outcomes Measurement Information System (PROMIS) Physical Function – EuroQol 5-Dimensional (EQ-5D-3L) questionnaire – Patient's Global Impression of Change (PGI-C) • Change from baseline in pain assessment using Brief Pain Inventory (BPI) short form
Evaluate safety and tolerability of AL102	• Frequency, duration and severity of treatment-emergent adverse events (TEAEs) and serious AEs • Time to treatment discontinuation due to TEAE

BICR: blinded independent central review; CR: complete response; DOR: duration of response; ORR: objective response rate; PR: partial response; PFS: progression-free survival; RECIST: Response Evaluation Criteria in Solid Tumors

(P 550) ON-TRK: A NON-INTERVENTIONAL STUDY OF LAROTRECTINIB IN PATIENTS WITH TRK FUSION SOLID TUMORS

Jonathan Trent, MD, PhD[1]; Silvia Novello, MD, PhD[2]; Ghazaleh Tabatabai, MD[3]; Francesco Passiglia, MD, PhD[4]; Domnita-Ileana Burcoveanu, MD, MSc[5]; Ricarda Norenberg, MSc[6]; Theodore W. Laetsch, MD[7], (1) Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, (2) Department of Oncology, San Luigi Hospital, University of Turin, Orbassano, Piemonte, Italy, (3) Department of Neurology and Interdisciplinary Neuro-Oncology, Center for Neuro-Oncology, Comprehensive Cancer Center, University Hospital Tübingen and the Hertie Institute for Clinical Brain Research, Eberhard Karls University Tübingen, Tübingen, Baden-Wurttemberg, Germany, (4) Department of Oncology, San Luigi Hospital, University of Turin, Turin, Piemonte, Italy, (5) Bayer HealthCare Pharmaceuticals, Inc., Basel, Basel-Stadt, Switzerland, (6) Chrestos Concept GmbH & Co. KG, Essen, Rheinland-Pfalz, Germany, (7) Children's Hospital of Philadelphia/University of Pennsylvania, Philadelphia, PA

Objective: Increasingly, both the health authorities and medical community are investing time and resources into evaluating real-world data on the efficacy and safety of precision biomarker-based treatments. Neurotrophic tyrosine receptor kinase (NTRK) gene fusions are oncogenic drivers in various cancers. Larotrectinib is a first-in-class, highly selective, central-nervous-system-active tropomyosin receptor kinase (TRK) inhibitor, approved for tumor-agnostic use in pediatrics and adults with TRK fusion cancer. Enrolling patients to the ON-TRK study (NCT04142437), a Food and Drug Administration and European Medicines Agency post-

marketing requirement for the approval of larotrectinib, is critical to continue to gain real-world knowledge about the treatment of patients with TRK fusion cancer and will contribute to the broader understanding of personalized cancer care. Here, we describe the ON-TRK clinical trial, which is currently in progress in patients with TRK fusion cancer.

Methods: ON-TRK is a non-interventional, open-label, global, prospective, multi-cohort study enrolling adult (aged ≥18 years) and pediatric (from birth to 18 years) patients with TRK fusion, locally advanced or metastatic solid tumors scheduled to receive larotrectinib (Figure 1). Patients with cancer harboring NTRK gene fusions, detected locally, are eligible. Patients who have had prior TRK inhibitor therapy and patients with NTRK genomic alterations other than fusions are excluded. Decisions on the dose and duration of treatment are at the discretion of the treating physician. The study objectives and variables are listed in Table 1. The primary endpoint is safety, including specific long-term safety parameters in pediatric patients. Secondary endpoints include efficacy (objective response rate, disease control rate, time to response, duration of response, progression-free survival, and overall survival), efficacy in subgroups (including age, NTRK gene and fusion partner, testing method, country/region, and prior therapy), long-term effects of larotrectinib in pediatric patients, and dosing patterns.

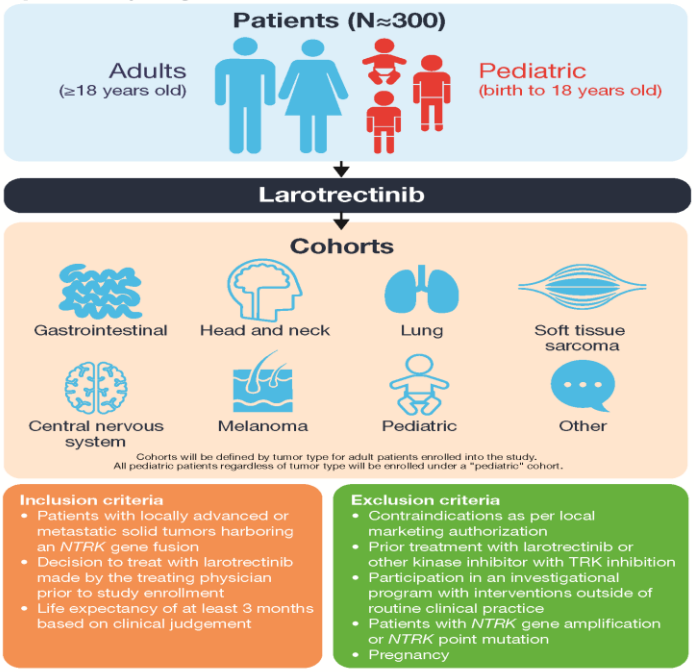
The effects of larotrectinib on growth and developmental milestones in pediatric patients will be reported, including change in height and weight from baseline, age at adrenarche for males and menarche for females, number and percentage of patients with abnormal Tanner stages, and abnormal neurological assessments. All variables will be analyzed using descriptive statistics.

Pediatric and adult patients will be followed for ≥5 years and ≥2 years, respectively, unless lost to follow-up, withdrawal, or death. Enrollment is ongoing worldwide and will include up to 300 patients, with ≥30 pediatric patients. As of May 2023, 70 patients have been enrolled, including 13 patients with either infantile fibrosarcoma or soft tissue sarcoma.

Results: .....N/A

Conclusion: .....N/A

Figure 1. Study design



NTRK, neurotrophic tyrosine receptor kinase; TRK, tropomyosin receptor kinase.

Table 1. Study objectives and variables

Objectives	Variables
Primary • Safety of larotrectinib in adult and pediatric patients with locally advanced or metastatic TRK fusion cancer	TEAEs in real-world practice conditions: • Incidence • Severity • Seriousness • Outcome • Causality assessment (ARs)
Secondary • Effectiveness of larotrectinib as per investigator assessment in the total study population as well as in patient subgroups (including, but not limited to: age, *NTRK* gene, *NTRK* gene fusion partner, testing methodology, country/region, and prior therapy [type and/or number of lines of therapy])	• Patient demographic and baseline characteristics • Disease history (including pathology report at baseline and subsequent reports as applicable) • Comorbidities • Method for detecting *NTRK* gene fusion (including testing report at baseline and subsequently as applicable) • ECOG, Lansky, or Karnofsky performance status • Tumor assessments (DCR, DoR, ORR, OS, PFS, TTR) per investigator assessment • Date of radiologic or clinical progression on larotrectinib
• Larotrectinib treatment patterns	• Larotrectinib use (initiation and termination dates, dosage, and dose modification)
• Long-term effects of larotrectinib on growth, developmental milestones, and sexual development in the pediatric cohort	• Developmental milestones, age at adrenarche or menarche (if applicable), Tanner scale
• Long-term effects of larotrectinib on neurologic outcomes in all patients	• Neurological examination • Laboratory examination data and date of death/last follow-up
AR, adverse reaction; DCR, disease control rate; DoR, duration of response; ECOG, Eastern Cooperative Oncology Group; *NTRK*, neurotrophic tyrosine receptor kinase; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; TEAE, treatment-emergent adverse event; TRK, tropomyosin receptor kinase; TTR, time to response.	

(P 551) PHASE II TRIAL OF GEMCITABINE AND NAB-PACLITAXEL IN PATIENTS WITH RECURRENT RHABDOMYOSARCOMA: A CLINICAL TRIAL IN PROGRESS FROM THE NATIONAL PEDIATRIC CANCER FOUNDATION

Aditi Dhir, MD[1]; Javier Oesterheld, MD[2]; Patrick Thompson, MD[3]; Thomas Badgett, MD[4]; Christine A. Pratilas, MD[5]; Bhuvana Setty, MD[6]; Masanori Hayashi, MD[7]; Matteo Trucco, MD[8]; Jonathan Metts, MD[9]; Damon Reed, MD[10]; Lars M. Wagner, N/A, MD[11], (1) University of Miami Miller School of Medicine, Miami, FL, (2) Atrium Health Levine Children's Hospital, Charlotte, NC, (3) University of North Carolina Health Care, Chapel Hill, NC, (4) University of Kentucky College of Medicine, Lexington, KY, (5) Johns Hopkins University, Baltimore, MD, (6) Nationwide Children's Hospital, Columbus, OH, (7) Children's Hospital Colorado, Aurora, CO, (8) Cleveland Clinic, Cleveland, OH, (9) Moffitt Cancer Center and Johns Hopkins All Children's Hospital, Tampa, FL, (10) Moffitt Cancer Center, Tampa, FL, (11) Duke Children's Hospital, Durham, NC

Objective: The combination of gemcitabine and docetaxel is commonly used in patients with recurrent bone or soft tissue sarcoma. Nab-paclitaxel is a nanoparticle albumin-bound taxane with less myelosuppression than docetaxel, and preclinical studies in pediatric sarcoma models have demonstrated activity in models of rhabdomyosarcoma (RMS). The combination of gemcitabine and nab-paclitaxel is now frontline therapy for pancreatic cancer, and responses in RMS patients have been reported with this combination in small retrospective series (Metts et al, Pediatr Blood Cancer 2017; Tian et al, BMC Cancer 2020). Therefore, we are currently conducting a prospective multi-institutional phase II trial to better define the activity of this combination in patients with recurrent RMS (clinicaltrials.gov identifier NCT02945800). The study is currently open at 14 pediatric oncology centers in the United States through the National Pediatric Cancer Foundation.

Methods: Patients age > or equal to 3 years with relapsed or refractory RMS who have measurable disease and adequate organ function are eligible. The trial uses a single arm, Simon’s two-stage design to identify an objective response rate of > 35%. Patients receive weekly gemcitabine 1000 mg/m2 and nab-paclitaxel 125 mg/m2 x 3 in four-week cycles, using the schedule approved for pancreatic cancer. Pegfilgrastim is used for all patients. The protocol was amended to specify that if myelosuppression is limiting after dose reduction, an alternate schedule may be used in which both drugs are administered weekly x 2 in three-week cycles. Responses are assessed by RECIST version 1.1. Correlative studies include immunohistochemical assessment of potential biomarkers of chemosensitivity (SPARC, CAV-1, hENT1), radiomic analysis of imaging studies, and identification of circulating tumor cells and tumor DNA in peripheral blood.

Results: N/A .....

Conclusion: N/A .....

(P 552) RANDOMIZED PHASE II TRIAL OF CABOZANTINIB COMBINED WITH PD-1 AND CTLA-4 INHIBITION VERSUS CABOZANTINIB IN METASTATIC SOFT TISSUE SARCOMA.

Brian Andrew Van Tine, MD, PhD[1]; Vanessa Eulo, MD[2]; Sasha Haarberg, PharmD, BCOP[1]; Jacqui USO Med, CCRP, CMA-C[1]; Ruth Gemima Nakiwala Katumba, MPH[3]; Jingqin Luo, MD[4]; Tyler Ruff, n/a[3]; Lindsey Kemp, BS[5]; Cristiam Moreno Tellez, MD[6]; Mia C. Weiss, MD[1]; Angela Hirbe, MD,PHD[7]; Christian F. Meyer, MD PhD[8]; Anthony D. Elias, MD[5]; Mark Diamond, MD, PhD[9]; Lee Hartner, MD[10]; Nam Bui, MD[11]; Kristen N. Ganjoo, MD[12]; Robert G. Maki, MD PhD[13]; Breelyn A. Wilky, MD[14], (1) Washington University School of Medicine, St. Louis, MO, (2) University of Alabama at Birmingham, Birmingham, AL, (3) Washington University School of Medicine, Saint Louis, MO, (4) Washington University School of Medicine, Department of Surgery, Division of Public Health Sciences, Saint Louis, MO, (5) University of Colorado Anschutz Medical Campus, Aurora, CO, (6) University of Colorado Anschutz Medical Campus, Denver, CO, (7) Washington University in St. Louis, St. Louis, MO, (8) Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, (9) University of Pennsylvania-Abramson Cancer Center, Philadelphia, PA, (10) Abramson Cancer Center, Philadelphia, PA, (11) Stanford University, Stanford, CA, (12) Stanford Cancer Center, Palo Alto, CA, (13) Memorial Sloan Kettering Cancer Center; Professor, Weill Cornell Medical College, New York, NY, (14) University of Colorado Cancer Center, Aurora, CO

Objective: Cabozantinib (C) has been combined successfully with either PD-1 or CTLA-4 inhibition in cancer clinical trials. The combination of Nivolumab (N) and Ipilimumab (I) previously was evaluated in soft tissue sarcoma (STS) and demonstrated activity as well as an acceptable safety profile. Given these data, we hypothesize that C in combination with both I and N is a therapeutic strategy that will be more effective than C alone in metastatic STS that lack translocations. Additionally, we hypothesized that C priming would enhance the efficacy of combination I and N at the time of crossover.

Methods: This is a 2:1 randomized phase 2 clinical trial evaluating the overall response rate (RR) of C 40mg orally daily in combination with I(1mg/kg IV) / N(3 mg/kg) for 4 doses Q3W and then maintenance N 480mg Q4W compared to C 60mg oral alone, with crossover. Secondary endpoints include progression free survival (PFS), disease control rate (DCR), RR in crossover, quality of life by FACT-G7, RR by iRECIST and safety. Correlative and biomarker analysis are preplanned. Key patient selection includes ECOG 0-1, 1-2 lines of prior therapy, and sarcomas that lack of translocations. The trial was balanced for leiomyosarcoma (LMS), liposarcoma and UPS.

Results: . N/A

Conclusion: . N/A

(P 553) A PHASE 1/2A OPEN-LABEL, DOSE-ESCALATION/DOSE EXPANSION SAFETY, TOLERABILITY AND PHARMACOKINETIC STUDY OF IMX-110 IN PATIENTS WITH ADVANCED SOLID TUMORS

Erlinda M. Gordon, MD[1]; Victoria Chua, MD, CLS[2]; Neal S. Chawla, MD[2]; Nadezhda Omelchenko, MD[2]; Sant P. Chawla, MD, FRACP[2], (1) Sarcoma Oncology Research Center, Santa Monica, CA, (2) Sarcoma Oncology Center, Santa Monica, CA

Objective: Background: IMX-110 (DOX/CUR Micelles) is a PEG-PE polymeric micelle encapsulating a Stat3/NF-kB/poly-kinase inhibitor, curcuminoid complex (CUR), and low-dose topoisomerase inhibitor, doxorubicin (DOX). Objectives: Primary: To determine the safety of IMX-110 administered intravenously in patients with advanced solid tumors; and to determine the maximum tolerated dose (MTD) and RP2D of IMX-110. Secondary: To determine the plasma PK profile of IMX-110 in treatment cycle 1; and to determine the efficacy of IMX-110 as assessed by ORR, DCR, PFS, DOR and OS. Exploratory: To assess the pharmacodynamic activity of IMX-110 with appropriate biomarkers.

Methods: Methods: Ten to 30 previously treated patients with advanced solid tumors will be enrolled in the Phase 1b component. Starting at 0.67 mg/m2 doxorubicin in IMX-110, the study has employed an accelerated dose escalation followed by the standard “cohort of three” design. The maximum tolerated dose based on doxorubicin (DOX-based) is defined as the highest safely tolerated dose, where not more than one patient experienced DLT, with the next higher dose level having at least two patients who experienced DLT. Phase 2 (n=40): Patients in the expansion phase will receive IMX-110 at the RP2D and may continue treatment until disease progression or unacceptable toxicity occurs. Statistical considerations: NIH CTCAE v5.0 and RECIST v1.1 will be used. There are three analysis populations: Safety, Efficacy in Evaluable Population and PK. Safety: All enrolled patients who receive any quantity of study treatment will be included in the Safety Population. Efficacy: All subjects who receive at least one full cycle of study treatment and have a follow-up RECIST assessment. PK: All subjects who receive study treatment and have sufficient plasma concentration for analysis of the PK parameters. Safety analysis: Incidence, severity and causality of AE and serious adverse events will be reported. Efficacy analysis: Summary tables presenting the number and proportion of responders and non-responders, together with the two-sided 95% Pearson-Clopper confidence intervals for response rates will be presented. The time to event endpoints, including DOR, PFS and OS will be analyzed by survival analysis method. In addition, the graphs of Kaplan-Meier estimates of survival functions will be presented. Descriptive statistics will be provided from available data from plasma (mandatory) and tissue (optional) samples collected and stored for biomarker analysis pre- and post-treatment. Nineteen patients have been dosed to date. Currently, Cohort 9 is being dosed.

Results: N/A This abstract is submitted under Trials in Progress

Conclusion: N/A This abstract is submitted under Trials in Progress

(P 554) FIVE-YEAR OUTCOMES OF A NATIONWIDE PROSPECTIVE CLINICAL TRIAL ON ACTIVE SURVEILLENCE IN PATIENTS WITH NON-INTRAABDOMINAL DESMOID-TYPE FIBROMATOSIS

Stefanie N. Hakkesteegt, MD[1]; Anne-Rose W. Schut, MD[1]; Milea J.M. Timbergen, MD, PhD[1]; Danique L.M. Van Broekhoven, MD, PhD[1]; Thijs van Dalen, MD, PhD[1]; Winan J. van Houdt, MD, PhD, MS[2]; Han Bonenkamp, Dr.[3]; Stefan Sleijfer, MD, PhD[1]; Dirk J. Grünhagen, MD, PhD[1]; Cornelis Verhoef, MD, PhD[1], (1) Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands, (2) Netherlands Cancer Institute/ Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands, (3) Radboud university medical center, Nijmegen, Gelderland, Netherlands

Objective: Active surveillance (AS) is currently recommended as initial management in desmoid-type fibromatosis (DTF). Results of the 3-year follow-up data of first prospective studies evaluating the AS approach as initial management in DTF, including the Dutch GRAFITI trial, were recently published [1]. These findings provided insights into the natural growth patterns of DTF over a 3-year period. The results illustrated either stable disease, regression or initial progression after three years of AS, however it remains unknown if (further) regression is still to be expected after a longer duration of AS. Moreover, data on long-term health-related quality of life (HrQoL) for patients with DTF is scarce. Patients with DTF frequently experience symptoms throughout the duration of the disease, negatively impacting their HrQoL. Results on HrQoL after two years of AS have recently been published [2], however long-term data after five years of AS will provide a more comprehensive understanding of the impact of tumor behavior and AS on patients HrQoL.
The aim of this study is to assess tumor behavior, the efficacy of AS and the HRQoL in patients with DTF after five years of follow-up.

Methods: In this multicenter prospective cohort study (NTR4714) adult patients with non-intra-abdominal DTF were followed during an initial AS approach. When AS was no longer feasible, patients could switch to active treatment (AT) if necessary. Tumor behavior was assessed by MRI according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. HRQoL during AS was evaluated by the EORTC QLQ-C30 at the moment of inclusion, 6 months, 1 year, 2 year and 5-year follow-up (Table 1). Cumulative incidence of the need to start an AT and progression-free survival (PFS) were calculated by the Kaplan-Meier method. Cox regression analyses were conducted to identify factors predictive for switch to AT. A multivariable linear mixed-effects model with random intercept was conducted to assess trends of HRQoL scores over time and to explore the effect of treatment strategy on HRQoL. At 3-year follow-up, results on tumor behavior, treatment choice (AS/AT) and HRQoL were analyzed and published (Schut et al. 2022). After 3-years of follow-up, patients could decide if they wished regular follow-up or follow-up on indication. The last included patient is expected to have completed the 5-year follow-up in November 2023. The 5-year data analysis are expected to be performed at the beginning of November 2023.

Results: Not applicable

Conclusion: Not applicable

References:
[1] Schut AW, Timbergen MJM, van Broekhoven DLM, van Dalen T, van Houdt WJ, Bonenkamp JJ, Sleijfer S, Grunhagen DJ, Verhoef C. A Nationwide Prospective Clinical Trial

on Active Surveillance in Patients With Non-intraabdominal Desmoid-type Fibromatosis: The GRAFITI Trial. Ann Surg. 2023 Apr 1;277(4):689-696.
[2] Schut AW, Timbergen MJM, Nasserinejad K, van Dalen T, van Houdt WJ, Bonenkamp JJ, Sleijfer S, Grünhagen DJ, Verhoef C, Husson O; Dutch Grafiti Group. Health-related Quality of Life of Patients with Non-intra-abdominal Desmoid-type Fibromatosis during Active Surveillance: Results of a Prospective Observational Study. Ann Surg. 2023 Jan 19;277(6):877–83.

Assessment	Enrollment	Year 1				Year 2		Year 3	Year 4	Year 5
Month		3	6	9	12	18	24	36	48	60
History and Physical examination	X	X	X	X	X	X	X	X	X	X
MRI-scan	X		X		X		X	X		
Ultrasound	X	X		X		X			X	X
QoL questionnaire	X		X		X		X			X

(P 555) A PILOT PHASE II STUDY TO EVALUATE THE SMALL MOLECULE TIGILANOL TIGLATE IN PATIENTS WITH ADVANCED SOFT TISSUE SARCOMA: CLINICAL TRIAL IN PROGRESS

Edmund K. Bartlett, MD; Duan Li, MD; Narasimhan Agaram, MBBS; Samuel Singer, MD, FACS; William D. Tap, MD; Ciara M. Kelly, MBBCh, Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Tigilanol tiglate is an epoxytigliane small molecule that induces tumor cell necrosis and tumor vascular disruption with potential to induce systemic immunity via immunogenic cell death when injected intratumorally. In a Phase I study, tigilanol tiglate was well tolerated (96% of adverse events were mild to moderate), and clinical activity was observed (four patients had a complete response and three patients had partial responses) in nine tumour types, with abscopal tumor responses in two patients (Panizza et al., 2019). One patient with angiosarcoma of the nasal bridge, who had been recommended for a total rhinectomy, had a complete response from a single injection of tigilanol tiglate 43 days post-injection. Three punch biopsies at 12 weeks revealed no residual tumor, the patient was disease free as assessed by CT scan at 25 months, and clinically disease free at 30.5 months post-injection (Panizza et al., 2019). Based on these promising early results, a trial has been designed to investigate the preliminary efficacy of tigilanol tiglate specifically in patients with advanced and/or metastatic

soft tissue sarcomas.

Methods: This is a single arm, open label pilot Phase II study of intratumoral tigilanol tiglate in 10 to 15 patients with advanced and/or metastatic soft tissue sarcoma. The primary objective is to evaluate the proportion of patients who achieve partial (≥30% response) or complete response of the injected tumor as assessed by ultrasound measurement. Secondary objectives are safety, tolerability, and pharmacokinetics. Exploratory objectives are to estimate tumor recurrence rates at 6 months and investigate correlative markers of tumor response in pre- and post-treatment biopsy and blood samples. Patients with both resectable and unresectable sites of disease are eligible. Patients with unresectable tumor(s) may receive repeat treatments of tigilanol tiglate (administered at 0.5 mL / 1.0 cm3 tumour, up to 3.6 mg/m2) at 28-day intervals up to a maximum of five treatments. Patients who are eligible for surgery will receive a single intratumoral treatment with tigilanol tiglate. Surgery may then be scheduled 28 days after injection, if it remains the most suitable treatment option at the discretion of the investigator. If the injected tumor shows a complete response (assessed as 100% reduction in tumour volume), patients will be monitored for recurrence. The trial is currently open to enrolment (NCT05755113). At the time of submission, one patient with a regionally recurrent myxofibrosarcoma has been treated, and a second patient is in screening.

Results: Not applicable

Conclusion: Not applicable

(P 556) EVALUATION OF PALBOCICLIB (IBRANCE®) IN COMBINATION WITH CHEMOTHERAPY IN PEDIATRIC PATIENTS WITH RECURRENT OR REFRACTORY EWING SARCOMA OR NEUROBLASTOMA

Theodore W. Laetsch, MD[1]; Margaret E. Macy, MD[2]; Fariba Navid, MD[3]; Brenda Weigel, MD[4]; Elizabeth Fox, MD[5]; Douglas S. Hawkins, MD[6]; Katherine A. Janeway, MD[7]; Jodi A. Muscal, MD[8]; Katarzyna A. Alicja Juszczak-Kosela, MD[9]; Zhe Zhang, MS[10]; Anderson B. Collier, III, MD[11], (1) Children’s Hospital of Philadelphia/University of Pennsylvania, Philadelphia, PA, (2) Children’s Hospital Colorado, University of Colorado School of Medicine, Aurora, CO, (3) Children’s Hospital Los Angeles, Los Angeles, CA, (4) Department of Pediatrics, University of Minnesota, Minneapolis, MN, (5) Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN, (6) Seattle Children's Hospital, Seattle, WA, (7) Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA, (8) Pfizer Inc, New York, NY, (9) Pfizer Inc, Warsaw, Mazowieckie, Poland, (10) Pfizer Inc, San Diego, CA, (11) Nemours Children's Health , Jacksonville, FL

Objective: Palbociclib is an orally administered, highly selective, reversible, small molecule inhibitor of cyclin-dependent kinases (CDK) 4 and 6. Cyclin D1 and CDK4 are critical for Ewing sarcoma (EWS) cell-line proliferation. Preclinical studies in EWS patient-derived xenograft models suggested potential antitumor benefit of adding palbociclib to irinotecan/temozolomide (IRN/TMZ) chemotherapy. Preclinical models also showed that palbociclib reduced growth of neuroblastoma (NBL) cell lines, induced G1 cell-cycle arrest, and inhibited the cyclin D1-Rb pathway. The phase 1 portion of the study for pediatric and young adult patients with recurrent/refractory (R/R) solid and central nervous system tumors established the recommended phase 2 dose (RP2D) of palbociclib in combination with IRN/TMZ and in combination with topotecan (TOPO) and cyclophosphamide (CTX). The study is now enrolling 2

cohorts: 1) a randomized, open-label, phase 2 cohort for patients with R/R EWS to determine whether the addition of palbociclib to IRN/TMZ prolongs event-free survival (EFS) and 2) a nonrandomized single-arm cohort for patients with R/R NBL to confirm the safety and evaluate the preliminary antitumor activity of palbociclib with TOPO/CTX.

Methods: R/R EWS Cohort Patients with R/R EWS are randomly assigned 2:1 to receive either palbociclib with IRN/TMZ or IRN/TMZ alone. The primary efficacy endpoint is EFS per investigator assessment. Secondary efficacy endpoints include EFS by independent review, objective response (OR), progression-free survival (PFS), and overall survival (OS). An interim futility analysis is planned to allow for early stopping of the study due to futility/no signal of activity based on investigator-assessed EFS. Safety and planned interim efficacy data will be assessed by an external Data Monitoring Committee. Key eligibility criteria include R/R EWS with at-least-evaluable disease in ≥1 site other than bone marrow, molecular confirmation of EWS R1-ETS or FUS-ETS rearrangement or availability of formalin-fixed paraffin-embedded tumor tissue sample for central testing, and age ≥2 and < 21 years at the time of study entry. Patients could have received prior IRN and/or TMZ if their disease did not progress on this regimen. Patients randomly assigned to the palbociclib with IRN/TMZ arm will receive palbociclib at the RP2D (75 mg/m2) orally (either as a capsule or oral solution) once daily on days 1–14 of each 21-day treatment cycle. IRN (50 mg/m2) will be administered intravenously (IV), and TMZ (100 mg/m2) will be given orally (IV if patient cannot swallow the TMZ capsule), both once daily on days 1–5. Patients randomly assigned to the IRN/TMZ-only arm will receive IRN/TMZ at the same doses and schedule. NBL Cohort Patients with R/R NBL will receive palbociclib with TOPO/CTX. The primary efficacy endpoint is objective response, as assessed by investigator using International Neuroblastoma Response Criteria (INRC). Secondary efficacy endpoints include duration of response, PFS, and OS. Preliminary antitumor activity will be evaluated using a modified Simon's 2-stage optimal design. Key eligibility criteria include R/R NBL, at-least-evaluable disease as defined by INRC, aged ≥2 and < 21 years at the time of study entry, and an adequate organ function and performance status. Patients could have received prior TOPO and/or CTX if their disease did not progress on this regimen. Prior treatment with a CDK4/6 inhibitor is exclusionary. Patients will receive palbociclib (75 mg/m2) orally once daily on days 1–14 of each 21-day treatment cycle. CTX (250 mg/m2) followed by TOPO (0.75 mg/m2) will be administered IV once daily on days 1–5. Concurrent radiotherapy or surgery for local disease control will be permitted. Both Cohorts Treatment will continue until disease progression, patient and/or legal guardian refusal, unacceptable toxicity, or up to 24 months of treatment, whichever occurs first. As of June 2023, 45 of 75 patients with R/R EWS have been enrolled in the randomized phase 2 EWS cohort and 4 of 21 patients with R/R NBL have been enrolled in the nonrandomized NBL cohort. Enrollment to both cohorts of the study is ongoing.

Results: Not applicable.

Conclusion: Not applicable.

(P 557) UNRAVELING THE ROLE OF LOCAL THERAPY FOR PATIENTS WITH OLIGOMETASTATIC SOFT TISSUE SARCOMA - A RETROSPECTIVE MULTICENTER TRIAL OF BAVARIAN UNIVERSITY HOSPITALS

Anton Burkhard-Meier, MD[1]; Matthias Grube, MD[2]; Markus Albertsmeier, MD[3]; Luc M. Berclaz, MD[1]; Sarah Consalvo, MD[4]; Dorit Di Gioia, MD[5]; Hans Roland Dürr, MD[6]; Michael Frühwald, MD[7]; Robert Grützmann, MD[8]; Felix Keil, MD[9]; Carolin Knebel, MD[4]; K. Martin Kortüm, MD[10]; Wolfgang G. Kunz, MD[11]; Ulrich Lenze, MD[4]; Norbert Meidenbauer, MD[12]; Sabine Semrau, MD[13]; Armin Wiegering, MD[14]; Martin Trepel, MD[15]; Lars H. Lindner, MD[5], (1) Department of Internal Medicine III, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (2) Department of Medicine III, University Hospital Regensburg, 93053 Regensburg, Germany, Regensburg, Bayern, Germany, (3) Department of General, Visceral and Transplantation Surgery, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (4) Department of Orthopaedic Surgery, School of Medicine & Klinikum rechts der Isar, Technical University of Munich, 81675 Munich, Germany, Munich, Bayern, Germany, (5) Department of Medicine III, LMU University Hospital, LMU Munich, Munich, Bayern, Germany, (6) Orthopaedic Oncology, Department of Orthopaedics and Trauma Surgery, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Germany, Munich, Bayern, Germany, (7) Swabian Children's Cancer Center, Children's Hospital, University Hospital Augsburg, 86156 Augsburg, Germany, Augsburg, Bayern, Germany, (8) Department of General and Visceral Surgery, University Hospital, Friedrich-Alexander- University Erlangen-Nuremberg, 91054 Erlangen, Germany, Erlangen, Bayern, Germany, (9) Department of Pathology, University Hospital Regensburg, 93053 Regensburg, Germany, Regensburg, Bayern, Germany, (10) Department of Internal Medicine II, University Hospital Würzburg, 97080 Würzburg, Germany, Würzburg, Bayern, Germany, (11) Department of Radiology, University Hospital, Ludwig-Maximilians-University Munich, 81377 Munich, Germany, Munich, Bayern, Germany, (12) Department of Medicine 5, University Hospital, Friedrich-Alexander-University Erlangen- Nuremberg, 91054 Erlangen, Germany, Erlangen, Bayern, Germany, (13) Department of Radiation Oncology, University Hospital, Friedrich-Alexander-University Erlangen-Nuremberg, 91054 Erlangen, Germany, Erlangen, Bayern, Germany, (14) Department of General, Visceral, Transplantation, Vascular and Pediatric Surgery, University Hospital Würzburg, 97080 Würzburg, Germany, Würzburg, Bayern, Germany, (15) Department of Hematology and Medical Oncology, University Hospital Augsburg, 86156 Augsburg, Germany, Augsburg, Bayern, Germany

Objective: In a metastatic stage prognosis of patients with soft tissue sarcoma (STS) remains poor with a median survival of 1-2 years under anthracycline-based chemotherapy as standard treatment. Local therapies (LT), mainly surgical resection and radiotherapy, are increasingly applied for patients with oligometastasis. The value of LT is insufficiently defined regarding the level of evidence. So far, only non-STS-specific phase II studies exist on this question. For patients with 1-5 metastases of different cancers a survival advantage was shown after addition of consolidative LT to a systemic therapy. Prognosis of patients with oligometastatic non-small cell lung and colorectal cancer could be improved by LT leading to its recommendation in the respective German S3 guidelines. Since there are no evidence-based standards for the use of LT in oligometastatic STS patients, the indication is determined situationally in multidisciplinary tumor boards based on criteria such as number, size, localization and resectability of metastatic lesions as well as disease progression and response to systemic therapy. The patients and STS subtypes who take the greatest advantage of LT have not been identified yet. To date, treatment algorithms for patients with oligometastatic STS are not established. Evaluation of our data will hopefully lead to a considerable gain of knowledge about the poorly understood

clinical picture of oligometastasis. Our goal is to establish evidence-based recommendations for LT in order to improve quality in the care of oligometastatic STS patients.

Methods: The present study is a retrospective, multicenter trial designed to evaluate the role of LT for metastatic STS. The project is funded by Bayerisches Zentrum für Krebsforschung (BZKF) and takes place in all 6 BZKF sites (LMU and TU Munich, University Clinics Erlangen, Regensburg, Würzburg and Augsburg). Included are all patients with metastatic STS who were recommended a metastasis-directed local ablative therapy in one of the BZKF tumor boards in the years 2017-2021. The expected patient number to be enclosed is around 350. The primary objective is to evaluate efficacy in terms of progression-free survival (PFS), local-progression-free survival (LPFS) and time to next treatment (TNT) after local therapy. Secondary objectives include overall survival (OS) and toxicity. In cooperation with all disciplines involved in sarcoma therapy a comprehensive case report form has been developed. In the context of this project the entire course of disease of this patient cohort including detailed information about all local and systemic therapies is recorded. In parallel, the implementation of a common prospective registry also offering opportunities for translational research has already been started.

Results: At date of upcoming CTOS meeting in November 2023, retrospective data will be evaluated and results can be presented. However, the prospective part will still be in progress.

Conclusion: At date of upcoming CTOS meeting in November 2023, a conclusion about the use of local ablative therapy in oligometastatic soft tissue sarcoma will be drawn based on our results.

(P 558) TRIAL IN PROGRESS: A PHASE 1/2 STUDY OF BRIGATINIB IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH ALK POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA OR INFLAMMATORY MYOFIBROBLASTIC TUMORS (ITCC-098)

Kim P. J Schellekens, MD[1]; Charlotte Rigaud, MD PhD[2]; Auke Beishuizen, MD PhD[3]; Michela Casanova, MD[4]; Gertruud Bakker, PhD[3]; Maaike Boonstra-Schelfhorst, MSc[3]; Laurence Brugières, MD PhD[2]; Rana Dandis, PhD[3]; Michael J. Hanley, PharmD PhD[5]; Alwin D. R. Huitema, Prof. dr.[6]; Samantha Lampron, n/a[5]; Yousif Matloub, MD[5]; Johannes H. M Merks, Prof. dr.[7]; Cecilia E. J Terwisscha van Scheltinga, MD[3]; Nicole Scobie[8]; Harm van Tinteren, Dr. ir.[3]; Florin Vranceanu, MD PhD[5]; Wilhelm Woessmann, Prof. dr.[9]; C. Michel Zwaan, Prof. dr.[1]; Reineke A. Schoot, MD PhD[3], (1) Princess Máxima Center for Pediatric Oncology and Erasmus MC, Utrecht, Utrecht, Netherlands, (2) Gustave Roussy Cancer Campus, Villejuif, Ile-de-France, France, (3) Princess Máxima Center for Pediatric Oncology, Utrecht, Utrecht, Netherlands, (4) Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Lombardia, Italy, (5) Takeda Development Center Americas, Lexington, MA, (6) Princess Máxima Center for Pediatric Oncology, The Netherlands Cancer Institute-Antoni Van Leeuwenhoek Hospital, University Medical Center Utrecht, Utrecht, Utrecht, Netherlands, (7) Princess Máxima Center for Pediatric Oncology and Division of Imaging and Oncology, University Medical Center Utrecht, University of Utrecht, Utrecht, Utrecht, Netherlands, (8) Zoé4life, Sullens, Vaud, Switzerland, (9) University Medical Center Hamburg-Eppendorf, Hamburg, Hamburg, Germany

Objective: Brigatinib is a second generation orally administered anaplastic lymphoma kinase (ALK) inhibitor, approved by FDA and EMA for the treatment of adult patients with ALK positive (ALK+) non-small cell lung cancer. In this population, brigatinib showed a superior efficacy,

tolerability, and central nervous system penetration at the recommended phase two dose of 180 mg once daily (qd) after a 7-day lead-in at 90 mg qd, compared to the first generation ALK inhibitor crizotinib. The most common ALK driven tumors in the pediatric and adolescent population are anaplastic large cell lymphoma (ALCL) and inflammatory myofibroblastic tumors (IMT). IMTs are rare solid tumors which are primarily treated with surgical resection. There is currently no standard of care approach for systemic treatment in case of relapsed/refractory (R/R) disease, or at primary diagnosis in case of metastasized or unresectable tumors, including if surgery is expected to be mutilating. The aim of this study is to assess the safety, efficacy, and pharmacokinetics of brigatinib monotherapy in children and adolescents with ALK+ ALCL and IMT.

Methods: The BrigaPED study is a non-randomized, open-label, multicenter, phase 1/2 trial of brigatinib in the context of a pediatric investigation plan (NCT04925609). In phase 1, brigatinib is evaluated in up to 3 dose levels according to the rolling six design. Brigatinib is orally administered once daily in 28-day cycles, with a maximum of 24 cycles. A 7-day lead-in phase was introduced before the start of cycle 1 to minimize the risk of early pulmonary toxicity based on prior experience in adults. Dose limiting toxicities (DLTs) are evaluated in the first 35 days of treatment, including the 7-day lead-in phase and the first cycle. The RP2D will be based on DLTs and pharmacokinetic data. Adverse events are graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Written informed consent and adequate organ function are required for study eligibility. The primary objective for phase 1 is to establish the recommended phase 2 dose (RP2D) of brigatinib monotherapy in children. Eligible patients for phase 1 are patients aged 1-18 years with: 1) newly diagnosed unresectable or metastatic, or R/R ALK+ IMT, 2) R/R ALK+ ALCL, including patients with persistent positive qualitative measurable residual disease (MRD) during frontline treatment, or 3) other ALK+ solid tumors. In phase 2 we will evaluate the efficacy of brigatinib monotherapy in patients aged ≥ 1 and < 26 years, with newly diagnosed unresectable or metastatic, or R/R ALK+ IMT (cohort B1, n = 28), or R/R ALK+ ALCL including patients with persistent positive qualitative MRD during frontline treatment (cohort B2, n = 22). A total of 36 sites in 16 countries across Europe and Israel are planned to be opened, of which the first opened in March 2022. To date, 8 patients were included in phase 1.

Results: Not applicable

Conclusion: Not applicable

(P 559) PROSPECTIVE PHASE II TRIAL OF PREOPERATIVE HYPOFRACTIONATED PROTON THERAPY FOR EXTREMITY AND TRUNCAL AND TRUNCAL SOFT TISSUE SARCOMA: THE PRONTO STUDY RATIONALE AND DESIGN

Emile Gogineni, DO[1]; Hao Chen, PhD[2]; Chen Hu, PhD[3]; Karim Boudadi, MD[4]; Jessica Engle, DO[4]; Adam S. Levin, MD[3]; Curtiland Deville, Jr., MD[4], (1) The Ohio State University Wexner Medical Center, Columbus, OH, (2) Johns Hopkins University School of Medicine, Bethesda, MD, (3) Johns Hopkins University School of Medicine, Baltimore, MD, (4) Johns Hopkins University School of Medicine, Washington, DC

Objective: Oncologic surgical resection is the standard of care for extremity and truncal soft tissue sarcoma (STS), often accompanied by the addition of pre- or postoperative radiation therapy (RT). Preoperative (preop) RT may decrease the risk of joint stiffness and fibrosis at

the cost of higher wound complication rates, occurring in 20-40% of patients. Late toxicity is also a common complication which affects quality of life, occurring in 10-35% of patients. Hypofractionated, preop RT has been shown to provide acceptable outcomes. A summary of prospective trials studying preop RT is provided in Table 1.

Proton beam therapy (PBT) provides the means to decrease dose to surrounding organs at risk and has not yet been studied in patients with extremity and truncal STS. We conducted an analysis comparing identical hypofractionated regimens of photon and proton RT as those used in this trial, showing significantly lower doses to skin, femur, and joint with PBT, as shown in Figure 1. Thus, PBT has the potential to provide lower rates of toxicity and wound complications than those associated with photon RT.

Our study titled “PROspective phase II trial of preoperative hypofractionated protoN therapy for extremity and Truncal soft tissue sarcOma (PRONTO)” represents the first trial evaluating the use of hypofractionated PBT for STS. We aim to prove the safety and efficacy of this approach and to compare our results to historical outcomes established by previous trials. Given the limited availability to proton centers, the short course of PBT may enable treatment for patients who would otherwise be limited when treating with several weeks of daily RT. We hope this trial will lead to increased referral patterns, offer benefits towards patient convenience and clinic workflow efficiency, and provide evidence supporting the use of PBT in this setting.

Methods: PRONTO is a non-randomized, prospective phase II trial evaluating the safety and efficacy of preop, hypofractionated PBT for patients with extremity and truncal STS. Patients who meet all inclusion and no exclusion criteria, as outlined in Table 2, will be enrolled.

A flow chart outlining the trial schema is provided in Figure 2. Treatment will consist of 5 fractions of PBT delivered every other weekday to a total dose of 30 Gy radiobiological equivalent. Clinical target volumes include gross tumor with a 3 cm margin longitudinally and 1.5 cm margin radially excluding natural barriers of spread, in addition to any surrounding edema seen on T2 weighted MRI, cropped 0.3 cm from skin. Oncologic surgical resection occurs within 2-12 weeks of completing PBT. Patients will be followed in the postoperative setting according to standard of care surveillance for STS.

The primary outcome is the rate of major wound complications within 90 days after surgery, as defined according to the National Cancer Institute of Canada Sarcoma2 (NCIC-SR2) Multicenter Trial. Secondary objectives include safety and tolerability (acute grade ≥3 adverse events), 1- and 2-year local recurrence- and distant metastasis-free survival rates, incidence of late grade ≥2 radiation toxicity (fibrosis, lymphedema, or joint stiffness), functional outcomes using the Musculoskeletal Tumor Rating Scale and the Toronto Extremity Salvage Score, quality of life assessed via Functional Assessment of Cancer Therapy-General forms, and pathologic response (complete response, positive margins, and percentage necrosis in comparison with pre-treatment biopsy).

A sample size of 36 evaluable patients will allow us to obtain a two-sided 90% exact confidence interval with a width less than 0.3 if the incidence is between 30% and 60%, based on those reported in previous prospective trials. Guarding against the potential that 10% of patients may be not evaluable, we plan to accrue 40 patients in total to provide 36 evaluable patients. Patients will be considered evaluable if they have received 1 fraction of PBT and completed surgery.

Results: N/A (Trial)

Conclusion: N/A (Trial)

Table 1. Prospective trials reporting outcomes after preoperative photon irradiation for extremity and truncal soft tissue sarcoma.

Study	Year	N	Dose / Fraction (Frequency)	BED (Gy)*	Radiation Technique	Time to Surgery after RT	% of Patients Receiving Chemotherapy	R0 Resection Rate (%)	Local Control	Toxicity	Wound Complications
Conventionally Fractionated											
O'Sullivan *et al*	2002	88	50 Gy / 25 fx (daily)†	75	3DCRT	3-6 wk	0	84	93% - 5 yr	32% / 18% / 15% G2 fibrosis / joint stiffness / edema	35%, 16% requiring reoperation
Kraybill *et al*	2006	64	44 Gy / 22 fx (daily)‡	66	Not reported	80 d	100	91	82% - 3 yr	83% G4, 5% G5	11% major wound complications
O'Sullivan *et al*	2013	59	50 Gy / 25 fx (daily)	75	IMRT	Not reported	0	93	88% - 5 yr	9% / 6% / 11% G2 fibrosis / joint stiffness / edema	31%, 8% requiring reoperation
Wang *et al*	2015	79	50 Gy / 25 fx (daily)§	75	75% IMRT, 25% 3DCRT	4-8 wk	0	76	94% - 3.6 yr	11% late G2	37%, 0% requiring reoperation
Lansu *et al*	2020	79[‖]	36 Gy / 18 fx (daily)[¶]	54	IMRT	39-53 d (median 44)	0	94	100% - 25 mo	14% late G2	22%, 17% requiring intervention
Hypofractionated											
Meyer *et al*	2014	16	28 Gy / 8 fx (daily)[#]	53	Not reported	6 wk	100	94	100% - 2 yr	88% G4	38% requiring reoperation
Kosela-Paterczyk *et al*	2014	272	25 Gy / 5 fx (daily)[**]	56	3DCRT	3-7 d	22	79	81% - 3 yr	15% late G2	32%, 7% requiring reoperation
Kubicek *et al*	2021	15[††]	35 (94%) or 40 (6%) Gy / 5 fx (q2d)	96-120	Cyberknife SBRT	29-83 d (median 41)	19	80[‡‡]	93%[‡‡] - 4.7 yr	27% late G1-2, 7% late G4	20% major wound complications
Gobo Silva *et al*	2021	18	25 Gy / 5 fx (daily)	56	3DCRT, IMRT	5-18 wk (median 9)[a]	100	83	95% - 29 mo	6% / 6% / 11% G2 fibrosis / joint stiffness / edema, 6% G3 joint stiffness, 6% G4 joint stiffness	33% major wound complications
Kalbasi *et al*	2022	52	30 Gy / 5 fx (daily)	75	76% IMRT, 20% 3DCRT, 4% electrons	2-6 wk (median 4)	12	82[b]	94% - 2 yr	16% late G2	32% requiring reoperation
Bedi *et al*	2022	32	35 Gy / 5 fx (q2d)	96	3DCRT, IMRT	19-67 d (median 41)	31	91	100% - 3 yr	22% G2 fibrosis, 13% G3 fibrosis	25% major wound complications

Abbreviations: N, patient number; BED, biologically effective dose; RT, radiation therapy; R0 resection, surgery with negative margins; fx, fractions; 3DCRT, 3D conformal radiation therapy; wk, week(s); yr, year(s); G4, grade 4; IMRT, intensity-modulated radiation therapy; mo, month(s); G3, grade 3; G2, grade 2; d, day(s); G5, grade 5; q2d, every other day; SBRT, stereotactic body radiation therapy; R1 resection, surgery with microscopically positive margins

* Assuming α/β of 4 Gy

† 11% of patients received postoperative boost for positive margins

‡ Split-course irradiation; postoperative boost was given to patients with positive margins

§ 15% of patients received postoperative boost for positive margins

‖ Only included patients with myxoid liposarcoma

¶ 1% of patients received postoperative boost for positive margins

6% of patients received postoperative boost for positive margins

** 8% of patients received postoperative boost for positive margins

†† 16 patients were enrolled and treated with SBRT. Oncologic and toxicity outcomes reported in this table include those from the 15 patients who underwent surgical resection

‡‡ 2 of the 3 patients with initial R1 resection underwent R0 re-resection. The 1 patient with R1 resection who was unable to undergo re-resection (due to medical issues) was the only patient who experienced local recurrence in this study, which occurred in the middle of the SBRT field 100 days after surgery

[a] Patients received 3 cycles of preoperative chemotherapy every 3 weeks. Radiation commenced with the start of cycle 2. Median time between the last cycle of chemotherapy and surgery was 6 weeks

[b] 5 of the 9 patients with initial R1 resection underwent R0 re-resection

Figure 1. Representative color wash images in axial (left), coronal (middle), and sagittal (right) planes comparing hypofractionated preoperative proton (top row) versus photon (bottom row) dose deposition for a lower extremity sarcoma, with the dose increasing as the color ranges from blue to green to red. As shown, the target receives similar coverage in the two plans, while the normal tissue outside of the target receives lower doses in the proton plan. Figure adopted from study by Thomas *et al.*

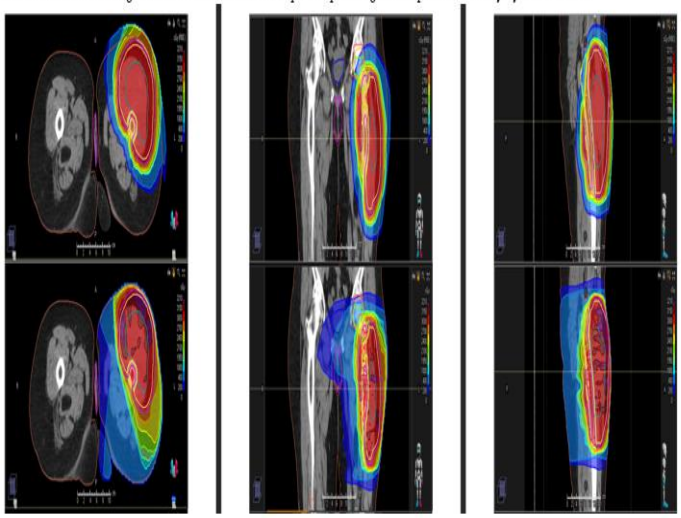
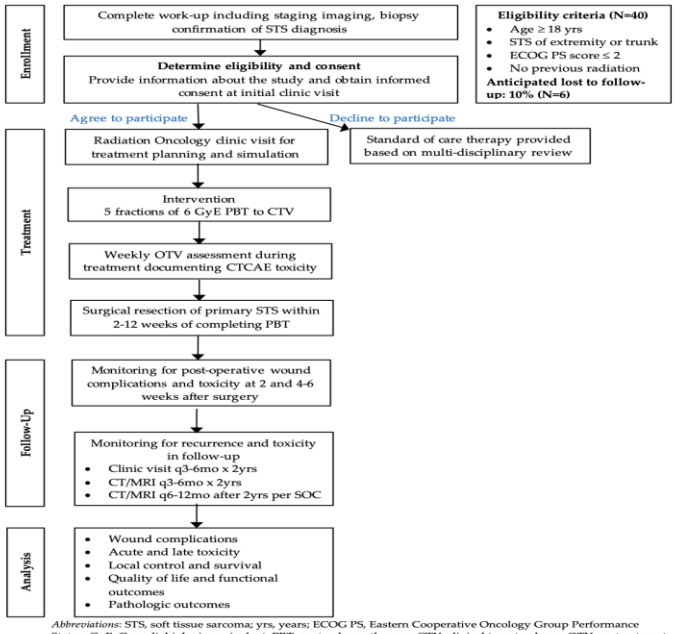


Table 2. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Written informed consent	History of prior local radiation therapy
≥18 years of age	Inability to tolerate treatment position for duration of simulation or treatment
Histologically confirmed, primary or locally recurrent extremity or truncal soft tissue sarcoma	Tumor originating in retroperitoneal location
Eastern Cooperative Group Performance Status ≤2	Patients planned for systemic therapy including chemotherapy, targeted agents, and immunotherapy
	Co-existing malignancy or treated malignancy in the last 2 years expected to limit life expectancy; does not include completely resected cutaneous basal cell carcinoma, squamous cell carcinoma, in situ breast or cervical malignancies, or other pathologies at the discretion of the investigators
	Confirmed pregnancy

Figure 2. Flow chart outlining trial schema, study procedures, follow-up, and planned analysis



Abbreviations: STS, soft tissue sarcoma; yrs, years; ECOG PS, Eastern Cooperative Oncology Group Performance Status; GyE, Gy radiobiologic equivalent; PBT, proton beam therapy; CTV, clinical target volume; OTV, on treatment visit; CTCAE, common terminology criteria for determining adverse events version 5.0; q3-6mo x2yrs, every 3-6 months for 2 years; CT, computed tomography; MRI, magnetic resonance imaging; SOC, standard of care

(P 560) REAL-WORLD MONITORING OF PHYSICAL ACTIVITY USING AN ACCELEROMETER AFTER LOWER LIMB SURGERY FOR A SARCOMA.

Lojain A. Kaki, MSc[1]; Stephen JG Taylor, MSc PhD[2]; Catherine Pendegrass, n/a[3]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[4]; Sherron Furtado, PhD[5], (1) University College London, london, England, United Kingdom, (2) University College London, Stanmore, England, United Kingdom, (3) University College London, Greater London, England, United Kingdom, (4) Royal National Orthopaedic Hospital, London, England, United Kingdom, (5) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom

Objective:
Introduction:
Sarcomas are a rare type of cancer that affects bone and soft tissues. There is heterogeneity in the representation of the disease, even the treatment varies from one individual to another. This makes it difficult to provide the best recommendation for aftercare and rehabilitation. The literature lacks information on what actual physical activity levels patients achieve in their homes and communities and how this relates to their quality of life.
Objectives:
The main aim of this study is to monitor the real-world physical activity levels patients achieve after lower limb surgery for a sarcoma. Other aims are to investigate how physical activity relates to demographics, clinical factors, and other outcomes. Describe which patients perform well compared to those suffering as well as relate it to their quality of life.

Methods: Patients were recruited as part of the STOMP (Stanmore Tumor Outcome Measure Project) at the Royal National Orthopedic Hospital NHS Trust, part of the London Sarcoma Service. They were asked to wear an accelerometer-based wearable monitoring device the ActivPAL ™ (PAL Technologies Ltd, Glasgow, Scotland), a triaxial accelerometer that measures the change in posture for seven consecutive days. Additionally, Patients were asked to fill out patient-reported outcome measures (PROMs) (Toronto Extremity Salvage Score (TESS), Modified Reintegration to Normal Living Index (mRNL), Quality of Life for cancer survivors(QoL CS), and EQ-5D). The ActivPAL provides the mean information as the number of steps and activity score (MET.h) which is the energy expenditure and the time spent in sitting, standing, cycling, and lying. The data was analyzed using Statistical Package for the Social Sciences (SPSS). Correlations were investigated using Spearman's test and predictors of poor outcomes using regression models.

Results: 23 patients with 12 bone and 11 soft tissue sarcomas participated. 13 were males and 10 were females. The median age of patients was 48 (IQR 40-63) years, and the time post-surgery was 4 (IQR 2-6) years. 21 underwent limb salvage surgeries and 2 amputations. The median scores of TESS was 76.8, QoL-CS 62.7, mRNL 90.9, and an EQ-5D index value 0.76. 73% of the patients led a sedentary lifestyle, wherein they spent more than 8 hours of their day sitting and lying. Only 2 patients did cycling. A positive significant correlation was seen between the type of sarcoma and PA (r= 0.441, p= 0.045*). Activity scores (MET.h) positively predicted QoL-CS (R2= 0.229, p= 0.021*), and so did number of steps (R2= 0.229, p= 0.021*). Sub-domains revealed that the number of steps positively predicted the psychological domain (R2= 216, p= 0.025*) and so did activity scores (MET.h) (R2= 0.235, p= 0.019*).

Conclusion: An accelerometer-based wearable provides an objective snapshot of sarcoma survivors' challenges at home. Sedentary behavior is dominant and physical activity predicts important outcomes regarding quality of life. Physical activity is therefore important to assess and manage using tailored rehabilitation interventions. Along with PROMs and wearables which

when combined can provide a detailed real-world perspective of patients' personal participation restrictions. This identifies high-risk and struggling patients and can trigger timely support and therapy interventions.

Table 1. Demographic Data.

	Percentage (%)	Average of Age
FEMALE	43.5	46.5
MALE	56.5	50.2
Total	**100.0**	**48.6**

Table 2. Minimum, maximum, and average of post-surgery assessment.

	MAX NUMBER OF YEARS POST-SURGERY	MIN NUMBER OF YEARS POST-SURGERY	AVERAGE NUMBER OF YEARS POST-SURGERY
FEMALE	9	1	5
AMPUTATION	9	3	6
LIMB SALVAGE SURGERY	8	1	5
MALE	29	1	6
LIMB SALVAGE SURGERY	29	1	6

Table 3. Type of Surgery Versus The Average Of Monitored Activities (Activity Score (MET.h), Number of Steps, Standing Time, Sitting Time).

	AVERAGE OF ACTIVITY SCORE(MET.H)	AVERAGE OF TOTAL NUM STEPS	AVERAGE OF STANDING TIME(M)	AVERAGE OF SITTING TIME(M)
AMPUTATION	**31.41**	**2733.00**	**129.08**	**509.79**
AKA	31.01	1605.00	143.86	596.23
BKA	31.80	3861.00	114.30	423.35
LIMB SALVAGE SURGERY	**33.07**	**6630.90**	**192.42**	**557.25**
BUTTOCK	32.35	4211.00	273.54	581.56
DFR	32.91	6151.78	190.79	581.79
DIAPHYSEAL FEMORAL REPLACEMENT	33.22	6394.00	274.57	446.83
EXCISION	32.93	6662.00	151.30	515.58
OTHER	35.45	12122.00	273.12	501.63
PELVIC	32.31	5049.50	189.58	533.39
PFR	33.51	8085.00	142.43	564.20
PTR	32.45	4675.00	242.28	568.98
THIGH	33.24	7380.00	159.97	528.01
TOTAL	**32.93**	**6291.96**	**186.91**	**553.12**

(P 561) EXPERIENCE OF PHYSICAL DISABILITIES AND CANCER-RELATED QUALITY OF LIFE AFTER SPINAL TUMORS- STUDY OF A NATIONAL SURVEY IN ENGLAND.

Harsha L. Khilnani[1]; Sherron Furtado, PhD[2]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[3]; Stephen JG Taylor, MSc PhD[4]; Catherine Pendegrass, n/a[5]; Hanny Anwar, n/a[3], (1) University College London, Harrow, England, United Kingdom, (2) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom, (3) Royal National Orthopaedic Hospital, London, England, United Kingdom, (4) University College London, Stanmore, England, United Kingdom, (5) University College London, Greater London, England, United Kingdom

Objective: 1) To investigate extent of disability and activity limitations experienced by patients with spinal tumors using the Sarcoma Assessment Measure (SAM) questionnaire, European Organization for the Research and Treatment of Cancer QoL Questionnaire (EORTC QLQ C30), and Toronto Extremity Salvage Score (TESS).
2) To investigate how the QoL varies with age, gender, tumor type and location, treatment type, time since diagnosis, and employment status.

Methods: A cross-sectional study design was performed to collect data from 25 patients diagnosed with spinal tumors. The participants completed the SAM questionnaire, EORTC QLQ C30, Global Rating Change Scale and TESS, which provided comprehensive assessments of disability levels, activity limitations, and QoL. The demographic and clinical information, including age, tumor type and location, treatment type, time since diagnosis, and employment status, were also collected similarly.
Domain scores for each of the questionnaires were calculated and statistical analyses including chi-square, Mann Whitney U, and Kruskal Wallis tests, will be utilized to determine whether any significant differences exist between disability levels and activity limitations across different demographic and clinical factors.

Results: The results show a wide variation in disability levels and activity limitations. A higher score for the SAM questionnaire indicates less deterioration. High scores for the functional and global scales indicate a better functioning and QoL, whereas high scores for the symptom scales indicate more intense symptoms. A higher score for TESS indicates increased difficulty in performing the tasks. Statistical analyses will be done to determine whether disability levels and activity limitations vary significantly based on age, gender, tumor type and location, treatment type, time since diagnosis, and employment status.

Conclusion: This study is being carried out to determine the degree to which spinal tumors impact disability levels and activity limitations in patients, encompassing physical, psychological, and social aspects. The multidimensional approach, involving the SAM questionnaire, EORTC QLQ C30, and TESS, will provide a comprehensive understanding of the patients' experiences. The findings will highlight the importance of considering demographic and clinical factors when evaluating disability and activity limitations. The unique challenges faced by individuals in this group, including the need for assistive devices, physical therapy, and emotional support, necessitate an integrated approach to rehabilitation. Understanding the unique features, correlations, and everyday difficulties faced by individuals with spinal sarcoma is essential for developing targeted interventions, improving treatment outcomes, and enhancing overall QoL. Building a strong support network and accessing comprehensive rehabilitation services are crucial for spinal sarcoma patients.

Table 1: Patient demographics

	Total No. (%)
AGE	
Mean (SD)	51.6 (19.17)
Adolescent young adults (18-39 years)	6 (24%)
Middle aged (40-64 years)	15 (60%)
Elderly (>65)	4 (20%)
GENDER	
Male	12 (48%)
Female	13 (52%)
SEXUAL ORIENTATION	
Heterosexual/straight	23 (92%)
Gay or Lesbian	1 (4%)
Bisexual	0 (0%)
Prefer not to say	1 (4%)
SITE OF DISEASE	
Upper limb e.g., arm	2 (8%)
Lower limb e.g., leg	6 (24%)
Pelvis	8 (32%)
Abdominal organ e.g., stomach	1 (4%)
Spine	25 (100%)
Chest	3 (12%)
Head and neck	7 (28%)
Other	6 (24%)
TYPE OF SARCOMA	
Soft tissue	9 (36%)
Bone	9 (36%)
Both	6 (24%)
TREATMENT RECEIVED	
Surgery alone	4 (20%)
Surgery and radiotherapy	5 (25%)
Surgery, radiotherapy and chemotherapy	7 (28%)
Radiotherapy alone	1 (4%)
No treatment	1 (4%)
Radiotherapy and chemotherapy	1 (4%)
Chemotherapy alone	2 (8%)
Chemotherapy and surgery	1 (4%)
None of these combinations	1 (4%)
Missing	2 (8%)
ETHNICITY	
English / Welsh / Scottish / Northern Irish / British	17 (68%)
Any other White background	2 (8%)
White and Black Caribbean	1 (4%)
Pakistani	1 (4%)
MARITAL STATUS	
Married/civil partnership	12 (48%)
In a long-term relationship/cohabiting (i.e living with partner)	4 (20%)
Divorced	2 (8%)
Single, - never been married and are not cohabiting	5 (25%)
Widowed	2 (8%)
EMPLOYMENT STATUS	
Employee in full-time job (30 hours plus per week)	4 (20%)
Employee in part-time job (30 hours plus per week)	1 (4%)
Self-employed full or part-time	1 (4%)
On a government supported training programme	0 (0%)
Full-time education at school, college or university	1 (4%)
Unemployed and available for work	0 (0%)
Permanently sick/disabled	5 (25%)
Wholly retired from work	8 (32%)
Looking after the home	2 (8%)
Doing something else	1 (4%)
TIME SINCE DIAGNOSIS	
<1 year	0 (0%)
2-5 years	3 (12%)

6-10 years	16 (64%)
>10 years	4 (20%)
Missing	2 (8%)
AMPUTATION STATUS	
Yes	1 (4%)
No	20 (80%)
Not applicable (did not have surgery)	4 (20%)
DISABILITY STATUS	
Completely disabled	1 (4%)
Severely disabled	2 (8%)
Moderately disabled	7 (28%)
Mildly disabled	5 (25%)
Not at all disabled	10 (40%)

Table 2: **PRELIMINARY CALCULATION OF QUESTIONNAIRE SCORES**

	MEAN SCORES ± SD	Median (95% C.I.)
SAM	67.41 ± 9.69	68.42
EORTC QLQ C30-		
Role Functioning	64.84 ± 38.81	67
Physical Functioning	63.4 ± 31.3	74
Cognitive Functioning	78.24 ± 20.74	84
Emotional Functioning	73.5 ± 18.82	75
Social Functioning	55.52 ± 38.76	67
Financial Functioning	29.32 ± 36.4	33.3
Symptoms-		
Fatigue	43.9 ± 27.85	44.3
Nausea & Vomiting	8.65 ± 21.5	0
Pain	39.96 ± 36.32	16.6
Dyspnoea	19.31 ± 25.3	0
Insomnia	39.97 ± 30.42	33.3
Appetite loss	19.98 ± 31.90	0
Constipation	19.98 ± 30.4	0
Diarrhoea	7.99 ± 22.1	0
Global health status	58.62 ± 21.44	66.6
TESS	71.69 ± 24.94	
Global Rating Change Scale		
Unchanged	13 (52%)	
Small change	8 (32%)	
Medium change	3 (12%)	
Large change	1 (4%)	

(P 562) **A PHASE 2, SINGLE ARM STUDY OF REGORAFENIB IN METASTATIC/UNRESECTABLE REFRACTORY SYNOVIAL SARCOMAS**

Sanal Fernandes, MD[1]; Sameer Rastogi, DM[2]; Shamim A. Shamim, MD[3]; Adarsh Barwad, MD[4]; Rambha Pandey, MD[5], (1) Department of Medical oncology, Dr BRAIRCH, All India Institute of Medical Sciences, New Delhi, Delhi, India, (2) Department of medical oncology, Dr BRAIRCH, All India Institute of Medical Sciences, New Delhi, Delhi, India, (3) Department of Nuclear Medicine, All India Institute of Medical Sciences, New Delhi, Delhi, India, (4) Department of Pathology, All India Institute of Medical Sciences, New Delhi, Delhi, India, (5) AIIMS, New Delhi, Delhi, India

Objective: Treatment options in advanced synovial sarcomas remain scarce. The VEGF pathway has been shown to drive proliferation in soft tissue sarcomas. This study aims to study the efficacy of the multikinase inhibitor regorafenib in advanced synovial sarcomas.

Methods: Methods:
Design: This a phase 2, Simon two-stage optimal design study

Sample size: 25 patients

Study centre: Dept of Medical Oncology, AIIMS, New Delhi, India
National Cancer Institute, Haryana

Study period: January 2022 to December 2023 with an additional one-year follow-up period

Participants: The study participants will include patients ≥16 years, with advanced synovial sarcomas and ECOG PS 0-2, that have progressed on two previous lines of therapy (one line being an anthracycline).

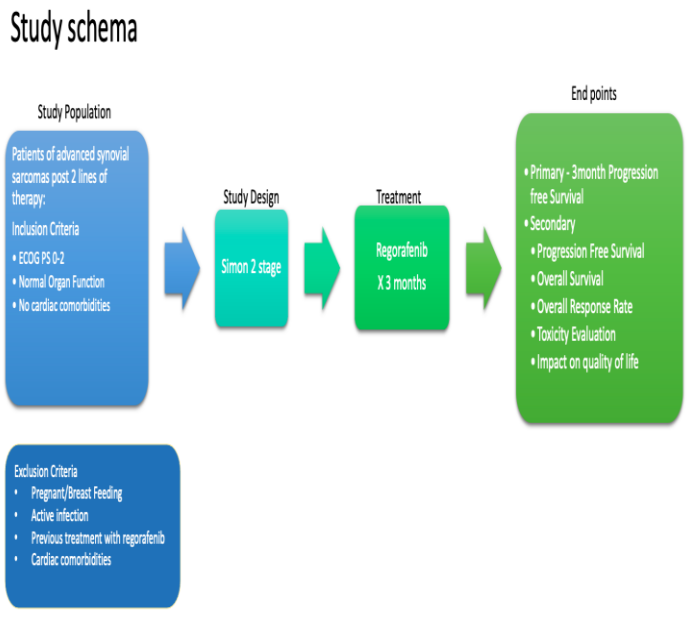
Intervention: Tablet regorafenib will be administered orally daily at a dose of 80 mg/ 120 mg on alternate days for a period of 3 months. Two sequential dose modifications will be allowed in case of grade 3 or higher toxicities.

Assessment:
Response assessment using RECIST 1.1 criteria will be undertaken at the end of 3 months. Patients who have not progressed at 3 months will be continued on the drug till clinical/ radiological progression with assessment every 3 months.
Toxicity evaluation using CTCAE v 5.0 will be done at monthly intervals. Quality of life assessment will be carried out at baseline and at 3 months using the EORTC QLQ C30 questionnaire.

End points:
Primary: 3-month progression-free rate
Secondary: a. PFS
b. OS
c. Overall response rate
d. Toxicity evaluation
e. Impact on quality of life

Results: N/A
The study has recruited 17 patients as of June 2023. Further accrual is ongoing.

Conclusion: NOT APPLICABLE



(P 563) BALANCE, GAIT AND BIOMECHANICAL COMPENSATIONS DURING DIFFERENT SPEEDS OF WALKING AFTER TREATMENT FOR DISTAL FEMORAL MUSCULOSKELETAL TUMOURS AND ASSOCIATED LIMITATIONS ON RETURN TO NORMAL LIVING

Tanvi Parikh, MSc[1]; Catherine Pendegrass, n/a[2]; Stephen JG Taylor, MSc PhD[3]; Craig Gerrand, MD, FRCS Ed (Tr and Orth)[4]; Sherron Furtado, PhD[5], (1) University College London, London, England, United Kingdom, (2) University College London, Greater London, England, United Kingdom, (3) University College London, Stanmore, England, United Kingdom, (4) Royal National Orthopaedic Hospital, London, England, United Kingdom, (5) Royal National Orthopaedic Hospital Nhs Trust, Greater London, England, United Kingdom

Objective: Surgery for distal femoral sarcomas frequently leads to reduced muscle strength and consequent gait compensations. These sequelae limit the patients' capability to resume daily activities, impacting their emotional well-being and predisposing them to falls. There is little literature on gait biomechanics at different speeds for lower limb sarcomas. However, everyday life involves walking at various speeds in an open environment, which requires dynamic balance. Hence, this study aims to assess the balance, gait and biomechanical compensations developed during different speeds of walking following distal femoral replacements. It also investigates the interaction of these factors with the physical struggles patients face when returning to normal living, as well as the effects on their functional activities, extent of disability, and quality of life.

Methods: This is a retrospective cross-sectional pilot study, a sub-study of Stanmore Tumour OutcoMes Project (STOMP) (Ethics approval: 21/WA/0027). Twenty-nine patients (17 females and 12 males) of mean age of 45±15.2 years who had an endoprosthetic replacement for distal femur sarcoma were enrolled into the study. The mean time elapsed since surgery was 7 years (1-29 years) with at least one year since surgery for all patients.

Data from 29 patients who filled out Patient-Reported Outcome Measures (Toronto Extremity Salvage Score, Re-integration into Normal Living, EQ-5D Five Level, Quality of Life Instrument-Cancer Survivor Version) were analysed along with datasets of 6 patients obtained from the Gait Real-Time Analysis Interactive Laboratory (GRAIL). GRAIL uses an instrumented treadmill with a 10-camera Vicon System and an interactive 180° surround screen. GRAIL was used as it enabled functional gait analysis at different speeds, simulated real-life conditions, and allowed continuous collection of data for one minute, thereby increasing its reliability. The maximum and minimum flexion values in the single support and swing phase were analysed for the three joints- hip, knee and ankle at 3 speeds (slow, mid-speed and fast). Kinetic data including joint moments and spatiotemporal parameters was also collected as studies have shown decreased moment demand at the knee and hip, reduced velocity, asymmetric stance and swing times, and increased step width in patients with a distal femoral endoprosthesis at normal speed (Carty et al., 2009, Okita et al., 2013).

At the time of writing this abstract, the data collection was complete. The data was analysed using Statistical Package for Social Sciences (SSPS) and Microsoft Excel, and multivariate nonparametric tests were run on the data. The results are pending and will be reported in the next 6 weeks.

Results: NOT APPLICABLE N/A

Conclusion: NOT APPLICABLE N/A

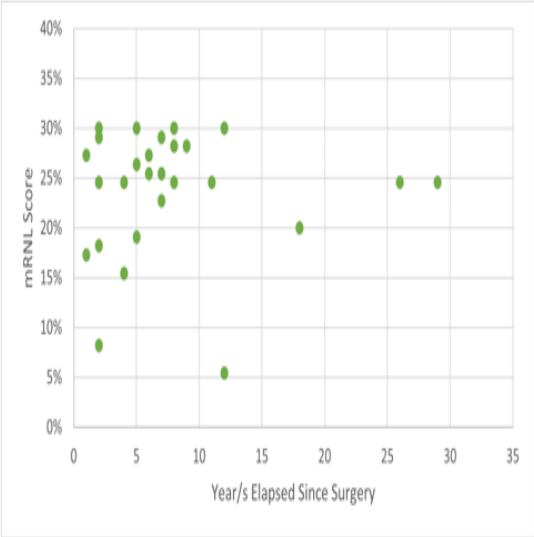


Figure 2 Scatter plot depicting mRNL index scores with respect to the number of years since surgery

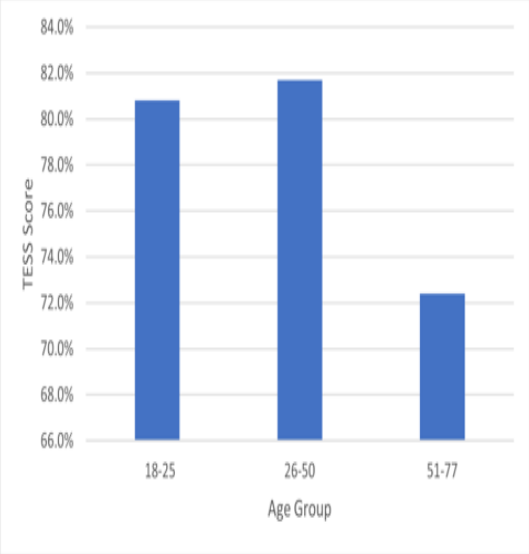


Figure 1 Bar graph comparing TESS score to classification of patients based on age

(P 564) A PILOT STUDY OF LYSO-THERMOSENSITIVE LIPOSOMAL DOXORUBICIN AND MAGNETIC RESONANCE-GUIDED HIGH INTENSITY FOCUSED ULTRASOUND FOR TREATMENT OF RELAPSED OR REFRACTORY SOLID TUMORS

Emily Leibold, BSE[1]; Pavel S. Yarmolenko, PhD[2]; Ann Liew, MS, CCRP[1]; Shari Thompson, BSN, RN, CPN, CPHON[1]; Karun Sharma, MD, PhD[1]; AeRang Kim, MD, PhD[1], (1) Children's National Hospital, Washington, DC, (2) The Sheikh Zayed Institute for Pediatric Surgical Innovation, Washington, DC

Objective: Current therapies for many pediatric solid tumors have reached maximal dose intensification and the acute and late effects of therapy are substantial. Magnetic Resonance-guided High Intensity Focused Ultrasound (MR-HIFU) is an innovative technology that enables precise thermal ablation by focusing ultrasound energy inside a tumor using an external applicator that is completely non-invasive and uses no ionizing radiation. In addition, pre-clinically, this therapy has been shown to amplify anti-tumor effects by causing a local inflammatory response, increasing antigen presentation and T-cell activation against tumor antigens. Lyso-thermosensitive liposomal doxorubicin (LTLD, ThermoDox®) is a special formulation that enables heat-activated release of doxorubicin. MR-HIFU provides precise and flexible control over local heating with hyperthermia (40-45ºC) and ablative temperatures (>55ºC). MR-HIFU is thus an ideal system to use with LTLD, as it can both trigger drug release with mild hyperthermia and prevent drug washout using ablation and associated vascular shutdown. The primary objectives of this study are to determine the response rate of targeted tumors to LTLD administered in combination with MR-HIFU hyperthermia followed by an ablation therapy in subjects with relapsed/refractory solid tumors, and to evaluate toxicity and safety of this combination therapy in subjects with refractory cancer. Exploratory objectives include evaluation of changes in pharmacodynamic immune biomarkers, radiographic response in non-target (not treated with HIFU) areas, and patient reported outcomes of pain and impact of pain on daily activities.

Methods: This is an open label pilot study evaluating LTLD with MR-HIFU hyperthermia followed by ablation (NCT04791228). Patients ≥12 years old with histologically confirmed relapsed/refractory measurable/evaluable solid tumors are eligible. Patients must have target lesions located in sites accessible to MR-HIFU. Adequate organ function, including cardiac function, is required. Prior anthracycline dosing must be ≤450 mg/m2. On day 1 of a 21-day cycle, LTLD will be administered intravenously over 30 minutes at the adult recommended phase 2 dose of 50 mg/m2. The infusion will be followed immediately (+/- 30 minutes) by one hour of MR-HIFU mild hyperthermia (40-45°C) and subsequent ablation (> 55°C) of the target volume. Therapy may continue for up to 6 total cycles if treatment is tolerated, patients have at least stable disease in treated lesions, and lifetime cumulative anthracycline dose of 600 mg/m2 is not exceeded. Outcome will be measured by imaging using modified RECISTv1.1 criteria. Patient reported outcome measurements of pain and quality of life as well as pharmacodynamic and immune markers in peripheral blood will be evaluated pre and post therapy to evaluate for any exploratory changes.

Results: N/A, trials in progress

Conclusion: N/A, trials in progress

(P 565) AN EARLY PHASE STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS, AND PRELIMINARY ANTITUMOR ACTIVITY OF PEEL-224 IN PATIENTS WITH ADVANCED SOLID TUMORS

Robert G. Maki, MD PhD[1]; Aleah Caulin, PhD[2]; Trent Fowler, MBA[2]; Sophia A. Howard, BA[3]; Ferro Nguyen[4]; Peng Guan, MS[4]; Ivan Alferiev, PhD[5]; Michael Chorny, PhD[5]; Garrett Brodeur, MD[5]; Debra Thoma Vallner, PhD, MBA[2]; Joshua Schiffman, MD[6], (1) Memorial Sloan Kettering Cancer Center; Professor, Weill Cornell Medical College, New York, NY, (2) Peel Therapeutics, Salt Lake City, UT, (3) Peel Therapeutics, New York, NY, (4) Children's Hospital of Philadelphia, Philadelphia, PA, (5) University of Pennsylvania Perelman School of Medicine; Children's Hospital of Philadelphia, Philadelphia, PA, (6) Peel Therapeutics; Huntsman Cancer Institute, University of Utah, Salt Lake City, UT

Objective: PEEL-224 is an improved topoisomerase 1 (TOP1) inhibitor, derived from camptothecin, for the treatment of patients with relapsed or refractory advanced solid tumors. Due to the limitations of currently available camptothecin-derived therapies (i.e., toxicities and chemoresistance), the clinical utility of TOP1-inhibiting drugs has not been fully realized in sarcoma. PEEL-224 has been engineered to overcome efflux-mediated drug resistance, supported by compelling in vitro and in vivo data. Nonclinical in vivo studies of PEEL-224 demonstrate sustained complete responses for more than 6 months in the most aggressive solid tumors, including Ewing sarcoma and rhabdomyosarcoma (Figure 1). We describe here an early phase trial of PEEL-224 in patients with advanced solid tumors that have relapsed or are refractory to prior systemic therapy.

Methods: Study PEEL-224-001 is a first-in-human, dose escalation, repeat-dose, multi-center (7 sites), open-label study to evaluate safety, tolerability, pharmacokinetics (PK), and preliminary antitumor activity of PEEL-224 in patients with advanced solid tumors. The primary objective is to determine the recommended phase 2 dose (RP2D) and to evaluate the overall safety and tolerability of PEEL-224. Secondary objectives include evaluation of the preliminary antitumor activity and characterization of the PK profile following PEEL-224 administration. Dose escalation is guided by the modified toxicity probability interval-2 (mTPI-2) design with a target toxicity rate of 25% and an acceptable dose limiting toxicity (DLT) interval of 20% to 30%. PEEL-224 is administered by intravenous infusion on Days 1, 8, and 15 of a 28-day cycle. The dose level increases between sequential cohorts of 3 or more patients according to mTPI-2 until a RP2D is determined. For each dose level, all patients must complete Cycle 1 before the decision to dose escalate the next cohort of patients is made. An additional arm of patients will be enrolled after dose escalation is completed to confirm the RP2D. Prior treatment with a TOP1 inhibitor is permitted and UGT genotyping is not required for enrollment since PEEL-224 is not metabolized via the glucuronidation pathway. Following this early-phase study, indications where PEEL224 has demonstrated clinically relevant activity and tolerability will be investigated in further studies. Other indications or treatment combinations will be explored where PEEL-224 may have promise. The study is registered with ClinicalTrials.gov as NCT05329103 and is currently accruing patients.

Results: Not applicable - - - - - - - -

Conclusion: Not applicable - - - - - - - -

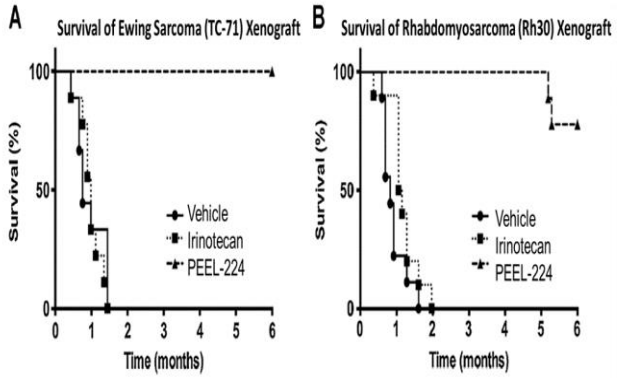


Figure 1. Survival of Ewing sarcoma (EWS) and rhabdomyosarcoma (RMS) xenografts treated with PEEL-224. Mice were treated with vehicle (saline), irinotecan (15 mg/kg/dose; equivalent to 10 mg/kg/dose SN38), or PEEL-224 (equivalent to 10 mg/kg/dose SN22; n = 10 for all) intravenously by tail vein once a week x 4 weeks. **A**, Survival of animals with flank xenografts of the EWS cell line TC-71 (chemoresistant) after 4 weeks of treatment. None of the PEEL-224 animals recurred. Log-rank (Mantel–Cox) test: PEEL-224 versus vehicle, $P < 0.0001$; PEEL-224 versus irinotecan, $P < 0.0001$. **B**, Survival of animals with flank xenografts of the alveolar (fusion-positive) RMS cell line Rh30 after 4 weeks of treatment. Two of the animals treated with PEEL-224 had a late recurrence, and one had a small, slow-growing tumor, but the 7 others were tumor free after 6 months. Log-rank (Mantel–Cox) test: PEEL-224 versus vehicle, $P < 0.0001$; PEEL-224 versus irinotecan, $P < 0.0001$. Figure adapted from Nguyen et al. (2020). "Structural Optimization and Enhanced Prodrug-Mediated Delivery Overcomes Camptothecin Resistance in High-Risk Solid Tumors." Cancer Research: canres.1344.2020.