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Allergy/asthma

F52. The Impact of Immunosenescence on Immune Responses in Older Individuals after Influenza Vaccination

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The goal of this study was to examine associations between age and immunosenescence markers (T-cell receptor excision circle [TREC] frequency, telomerase [TERT] expression, CD28 expression on T-cells, and the CD4+/CD8+T-cell ratio) and vaccine-induced immune responses (HAI/VNA/B-cell Elispot) and identify immunosenescence-associated differences in gene expression (mRNA-Seq) and gene regulation (miRNA-Seq/DNA methylation) in older adults (159 healthy subjects; 50-74 yo; 61.6% female; 98.7% Caucasians) receiving TIV (Fluarix) vaccination. A positive correlation (r=0.49) was observed between the %CD4+CD28- and %CD8+CD28- cells and a negative correlation (r=-0.33) was found between TREC and %CD8+CD28- cells. The peak antibody response (HAI/VNA titer at Day 28) was negatively associated with age (r=-0.2, p=0.04). TERT activity was positively correlated with the memory B-cell response at Day 28 compared to baseline (p=0.025). TREC levels were positively correlated with the baseline and early (Day 3) influenza A/H1N1-specific B-cell response (p=0.04 and 0.035, respectively). We identified an interconnected set of gene expression pathways (antigen processing/presentation/MAPK/mTOR/TCR/BCR/calcium signaling) that were associated with immunosenescence markers, such as TERT, TREC, %CD4+CD28- and %CD8+CD28- cells. We discovered several miRNAs (miR-320a,b,d) associated with chronological age, %CD4+CD28- cells and CD4+/CD8+T-cell ratio (p< 0.003). We found several CpGs (KLF14/TSPAN33) associated with age within the genes controlling immune function. We found that immune cell composition/gene expression/CpG methylation may provide distinct information for the prediction of humoral immune response outcomes. Our data suggest that influenza-specific immunity is significantly influenced by age, and that specific markers of immunosenescence are correlated with different humoral immune response outcomes observed after vaccination in older individuals.

F96. A novel role for long noncoding RNAs in airway responses to type 2 cytokines.

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During type 2 inflammation, such as in allergic asthma, IL13 signals through STAT (Signal Transducer and Activator of Transcription) proteins to drive pathophysiological changes in the airway epithelium, including increased mucus production, goblet cell metaplasia, and loss of ciliated cells. How these changes are coordinated is poorly understood. Long non-coding RNAs (lncRNAs) do not encode proteins; rather, some produce functional RNA transcripts that are powerful regulators of cellular identity and function. Using RNA-seq we identified a lncRNA that is induced directly by STAT6 following IL-13 activation. Using CRISPR/Cas9, ATAC-Seq and single-cell RNA-Seq, we have determined that this lncRNA coordinates the response of the airway epithelium to IL13 and hypothesize it provides a

mechanistic link between IL13 signaling and lung pathology seen in asthma. The work highlights a new role for lncRNAs as central regulators of airway epithelial cell biology, and as potential therapeutic targets for asthma.

F167. An Endothelial MicroRNA-1–regulated Network Controls Eosinophil Trafficking in Asthma and Chronic Rhinosinusitis.

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Background: Airway eosinophilia is a prominent feature of asthma and chronic rhinosinusitis, (CRS) and endothelium plays a key role in regulating eosinophil trafficking. MicroRNA-1 (miR-1) is the only miRNA reported to be altered in the endothelium of murine asthma models.

Objective: We sought to determine the specific role of endothelial miR-1 in regulating the eosinophilic response.

Methods: We measured miRNA and mRNA expression by quantitative RT-PCR. We used ovalbumin and house dust mite models of asthma and overexpressed miR-1 through delivery of a lentiviral vector or induction of an endothelial-specific transgene. Tissue eosinophilia was quantified by Congo red and anti-eosinophil peroxidase staining. We measured eosinophil binding using Sykes-Moore adhesion chamber. Target recruitment to miRNA induced silencing complex (miRISC) was assessed by anti-Argonaute2 RNA immune-precipitation. Surface p-selectin was measured by flow cytometry.

Results: Serum miR-1 levels had inverse correlations with sputum eosinophilia, airway obstruction, and number of hospitalizations in asthma patients. MiR-1 also had an inverse correlation with eosinophilia in CRS tissue samples. IL-13 decreased miR-1 levels in human lung endothelium. Endothelial-specific overexpression of miR-1 reduced airway eosinophilia and asthma phenotypes in murine models and inhibited IL-13-induced eosinophil binding to endothelial cells. MiR-1 recruited P-selectin, thymic stromal lymphopoietin, eotaxin-3, and thrombopoietin receptor to the miRISC. Expression of these target genes in our CRS and asthma cohorts correlated inversely with miR-1 levels. Further, miR-1 decreased surface P-selectin levels in the IL-13-stimulated endothelial cells.

Conclusion: Endothelial miR-1 regulates eosinophil trafficking in allergic airway inflammation. MiR-1 has therapeutic potential in asthma and CRS.

F182. Distinct gut microbiota profiles during active oral immunotherapy of peanut allergic subjects and healthy controls.

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Background: The prevalence of food allergy (FA) is increasing and has been associated with changes in gut microbiota. Oral Immunotherapy (OIT) is a widely studied immunotherapy to FA however the roles of gut microbiota during OIT are poorly understood. In this pilot study, we sought to study the gut microbiome alterations from peanut allergic participants who underwent peanut OIT and compared with heathy controls.
Methods: Stool samples were collected from peanut allergic participants at baseline, during OIT, and after a 13-week phase of withdrawal (n=16) or a daily dose of 300 mg (n=10) or from healthy controls (n=39). Shotgun metagenomic sequencing was performed and analyzed for taxonomic classifications. Alpha diversity was calculated and compared by Wilcoxon signed-rank test. Beta diversity was analyzed by Principal Coordinates Analysis (PCoA), then the difference was compared by Permutational Multivariate Analysis of Variance.
Results: The PCoA analysis revealed significant and independent separations between peanut allergic participants at baseline and healthy controls ($p < 0.001$) as well as for the ages of the individuals ($p < 0.001$). During OIT, significantly increased alpha diversities measured by richness ($p < 0.05$), Shannon index ($p < 0.05$), and Pielou's evenness ($p < 0.05$) were observed in seven participants at week 52 of OIT compared to baseline.
Conclusion: The PCoA analysis suggested different microbial communities in peanut allergic and healthy individuals. During OIT, the significantly increased alpha diversity at week 52 compared to baseline might suggest that actively increasing doses of peanut during OIT induces an increase gut microbial diversity in our pilot study.

F258. Characterization of the immune response in autoimmune chronic urticaria patients and the effect of anti-IgE treatment

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Introduction

The pathogenic mechanisms involved in chronic urticaria (CU) are complex. In approximately 40% of the patients, an autoimmune pathogenesis IgG mediated is involved and an association with autoimmune thyroid disease was reported. Anti-IgE treatment with omalizumab is used to treat CU patients leading to clinical improvement. The relationship between omalizumab and immune response involved in CU is still unknown.

Aim

To characterize the immune response of patients with CU and associated autoimmunity.

Methods
We analysed 49 patients diagnosed of CU, 22 in treatment with omalizumab and 27 with non-immunomodulatory drugs (NID). We performed flow cytometry immunophenotyping of T cell subpopulations and indirect Basophil Activation Test (BAT; to detect IgG autoantibodies anti-IgE). Total IgE and anti-thyroid antibodies were measured.

Results
Eight patients (18%) gave a positive result in indirect BAT test, 3 of them under omalizumab treatment. Patients with positive BAT had higher levels of activated $CD4^+$ T lymphocytes. Moreover, patients with anti-thyroid antibodies showed higher percentages of effector memory $CD8^+$ T cells than patients without anti-thyroid antibodies.
Regarding the effect of omalizumab in T cell subsets, patients under treatment showed a lower percentage of activated $CD4^+$ T lymphocytes but a higher percentage of central and effector memory Th1 and Th2 subpopulations

Conclusions
In this preliminary study, distinct patterns of T cell subsets were found in patients with autoantibodies anti-IgE and anti-thyroid. Furthermore, relevant changes in memory T cell subpopulations had been observed in patients under omalizumab. Further investigation is needed to correlate these changes with treatment response.

F266. Rate of Dose-related Adverse Events Over Time During a Randomized Phase 2 Peanut Oral Immunotherapy Study

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Rationale: Oral immunotherapy (OIT) for the treatment of peanut allergy appears promising, however the safety over time is unknown. OIT-related adverse events (AEs) during peanut OIT in a multi-year study were evaluated to better understand long-term safety.

Methods: 120 participants aged 7-55 years with confirmed peanut allergy enrolled in a double-blind, placebo-controlled, phase 2 study of peanut OIT. Participants were randomized to escalate to and maintain 4000 mg peanut protein (n=95) or placebo (n=25) daily over 104 weeks. The per-person AE rate was calculated by dividing the number of AEs per year by the number of doses taken each year. Differences in per-person median AE rates between groups of interest were evaluated using Kruskal-Wallis rank sum test.

Results: The overall AE rate significantly decreased from 0.50 in year 1 to 0.14 in year 2 ($p< 0.0001$), with a significantly greater reduction in AE rates from year 1 to year 2 in the peanut arm compared to placebo (-0.22 vs 0.00, $p=0.0043$). The rate of moderately severe AEs significantly declined from year 1 to year 2 in the peanut arm (0.09 vs 0.00, $p=0.093$). Participants reporting AEs related to accidental peanut ingestion decreased between year 1 (9%) and 2 (2%) in the peanut arm (Fisher's exact test $p=0.06$), while placebo did not change (12% to 16%, $p=1.00$).

Conclusions: Our findings show that the safety profile of peanut OIT improves over 3 years of dosing in this large, phase 2 study.

F267. A Positive Perception of Treatment in a Long-term Follow-up Study in Food Allergic Participants Undergoing Oral Immunotherapy

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Rationale: The diagnosis of food allergy can have a significant negative impact on the quality of life of patients and their families. Although oral immunotherapy (OIT) has been an effective treatment of food allergy, its effect on quality of life remains unknown.
Methods: We surveyed patients and/or caregivers with food allergy who have previously been treated with OIT at our research center. To assess caregiver perception and patient quality of life the Food Allergy Quality of Life Questionnaire (FAQLQ) and Food Allergy Quality of Life - Parental Burden Questionnaire (FAQL-PB) forms were used, and results for pre-OIT and post-OIT were compared.
Results: A total of 83 patients and/or caregivers responded to the survey. For patients enrolled between ages 0-12, there was statistically significant improvement in quality of life (QoL; pre-OIT mean 3.4 $\pm$3, post-OIT mean 2.0 $\pm$ 1.3, $p < 0.0001$, N=26) and parental burden (PB; pre-OIT 3.5 $\pm$ 1.5, post-OIT 1.6 $\pm$ 1.6, $p < 0.0001$, N = 30). In addition, 87% of all respondents felt "extremely positive" or "positive" when asked to rank the burden of treatment of OIT dosing (N=83).
Conclusions: Both QoL and PB improved significantly following treatment with OIT revealing a positive perception of OIT among food-allergic participants and their families.

F268. Characterization of Longitudinal Eosinophilic Gastrointestinal Response During Peanut Oral Immunotherapy in a Phase 2 Randomized Controlled Trial

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Rationale: Gastrointestinal side effects are common during oral immunotherapy (OIT) and the development of eosinophilic esophagitis (EoE) is a potential complication. In a randomized controlled trial involving peanut OIT, we characterized eosinophilic gastrointestinal responses over time.

Methods: Twenty adult subjects with peanut allergy were randomized to peanut OIT (n=15) and placebo (n=5). Serial gastrointestinal biopsies were obtained at baseline (0 weeks), following dose escalation (n=10, 52 weeks), and maintenance (n=12, 104 weeks). Endoscopic findings were characterized using the EoE endoscopic reference score (EREFS). Biopsies were assessed for eosinophils per high-power field (eos/hpf) and other pathologic features using EoE Histologic Scoring System (EoEHSS). Immunohistochemical staining for eosinophil peroxidase (EPX) was performed and quantified using automated image analysis.

Results: At baseline, no subjects reported gastrointestinal symptoms; however, 3 participants had ≥15 eos/hpf (esophagus) and all subjects had dilated intercellular spaces. OIT induced significant eosinophilic inflammation at 52 weeks in the proximal, middle, and distal esophagus; whereas no significant changes were seen in the placebo arm. These changes corresponded with significant increases in EoEHSS scores and EPX deposition. Four subjects (57%) had new-onset or worsening eosinophilia (≥15 eos/hpf) during OIT and one met clinicopathologic criteria for EoE. Three OIT subjects (43%) also crossed histologic thresholds for eosinophilic gastritis and/or duodenitis. In most, OIT-induced gastrointestinal eosinophilia (GE) resolved by the end of maintenance therapy and symptoms were not clearly associated with GE.

Conclusions: Our findings show that peanut OIT induces transient GE, and less commonly EoE, that is not always associated with gastrointestinal symptoms

F338. A systems pharmacology model approach to predict impact of anti-ST2 therapy in severe asthmatics

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To address the challenges in developing novel treatments for severe asthma, we have developed a quantitative systems pharmacology (QSP) model of asthma with explicit consideration of the mechanisms impacted by pharmacological interventions. The model captures the essential aspects of eosinophilic asthma and includes key modules such as epithelial activation; eosinophilopoiesis and recruitment; basophil, mast cells, ILC2s, and dendritic cells dynamics; adaptive immunity (Th2 and B cells); IgE production; degranulation; cytokines/chemokines/granule proteins; IgE; and clinical endpoints including FeNO and FEV1.

These modules are represented mathematically and calibrated to match available data for clinical endpoints. It is able to reproduce baseline biomarkers profiles in severe and healthy populations and their changes based on clinical studies of several therapies targeting a range of asthma related pathways including anti-IL13, anti-IL5, anti-IL-4Ra, anti-TSLP, and anti-IL-33. Variability in parameters is captured using a virtual population approach for severe asthmatics on ICS and LABA background.

Using this asthma QSP platform model, we investigated the putative effects of anti-ST2 therapy in our virtual population. We predict substantial reductions in FeNO and blood eosinophils, and increases in FEV1. These predictions are bolstered by simulations of anti-IL-33 which were found to agree with recently reported data for Etokimab. The predictions further suggest that anti-ST2 has similar impact as Tezepelumab (anti-TSLP), based on reported biomarker changes. We further analyzed the impact of the relative strength of ST2 in modulating granulocyte and type 2 cytokine-related pathways and predict a range of biomarker changes dependent on anti-ST2's activity on these two pathway groups.

F401. Multicohort Analysis of Bronchial Epithelial Cell Gene Expression Classifies Asthma from Healthy

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Asthma is a heterogeneous syndrome characterized by coughing, wheezing, airway obstruction, and shortness of breath varying over time. Research over the past decades has identified the type 2 (T2) helper T cells pathway in asthma and led to the development of targeted therapies. However, studies have identified further subsets of asthma for who the underlying biology and immune dysregulation remains poorly understood. This is consistent with observed phenotypes among people with asthma. Therefore, there remains an unmet critical need to further elucidate mechanisms for the distinct pathobiology in subpopulations of asthma to provide molecular phenotyping for risk stratification, new therapeutic development, and precision clinical management. Our objective is to identify a generalizable gene signature in from bronchial epithelial cells to classify asthma and identify genes involved in the underlying biology within asthma. We performed an integrated multicohort analysis of transcriptome data from bronchial epithelial cells across 6 data sets composed of 395 samples. We used 4 of these data sets for discovery and identified a 10 gene signature (false discovery rate < 1%, effect size > 0.2, leave-one-dataset-out cross validation). We validated this signature in the two data sets excluded from the discovery process, which accurately distinguishes subjects with and without asthma (receiver-operating characteristic curve with AUC of 0.8-0.82). Our results demonstrate the robust results generated by multicohort analysis to classify asthma and supports previous work identifying subsets of

asthma exist with distinct underlying biology. These methods will guide future investigations into asthma biology.

TH10. Peripheral Blood Immunome in Cow's Milk Specific Allergy Patients Revealed by High Dimensional Mass Cytometry

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Cow's milk allergy is a general health problem and most common food allergies affecting infants and young children. The majority of children spontaneously develop tolerance whereas a small percentage have persistent allergy to cow's milk. A thorough understanding of the Immunome in allergic patients may provide more insight on mechanisms involved in the development of tolerance. The current study employed mass cytometry based high dimensional analysis of peripheral blood mononuclear cells from allergic patients (n=10) and healthy controls (n=9). Using a 37 marker (surface and intra-cellular) panel, major immune cell lineages and subsets were identified. Unsupervised clustering analysis revealed significant differences in T and NK cell subsets in patients. Correlation network analysis of immune cell subsets displayed an dysregulated immune architecture in allergic patients. Higher modularity and interactions between CD4 and CD8 T cells subsets were observed in allergic patients. Correlation studies with cow's milk specific IgE in plasma and frequencies of immune subsets, highlighted differences in allergic patients as compared to healthy controls. Frequency of regulatory T cells was positively correlated with plasma IgE levels. Similarly, total plasma IgE levels, correlated differently with specific immune cell subsets in allergic patients. Overall, this study presents a detailed analysis of peripheral blood immunome in allergic patients and reports significant differences in specific immune cell subsets as compared to healthy controls. Results presented here have implications in understanding mechanisms involved in pathogenesis and tolerance development and is of prognostic value.

TH55. B cell-specific Plexin B2 plays a functional role in early B-cell development and antigen-specific responses

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B cell responses are regulated by a multitude of signals from soluble factors and cell-cell interactions. Plexin B2, a transmembrane protein implicated in axonal guidance and cell migration in non-B cells, is significantly upregulated on germinal center (GC) B cells. Despite the importance of GC responses in

mounting an effective humoral immune response, the function of Plexin B2 in B cells remains unknown. Here, we sought to evaluate the function of B cell-specific Plexin B2 expression on B cell development and GC responses. To evaluate the function of Plexin B2 in B cells, we generated B cell-specific Plexin B2 conditional knockout mice (CD19Cre/+PlexinB2fl/fl). Baseline phenotyping of conditional knockouts revealed a significant decrease in the frequency of B cell precursors and mature B cells in the bone marrow and spleen, respectively. Naïve conditional knockout mice further exhibited disorganized splenic follicles and significantly lower plasma IgM. Ovalbumin (OVA)-sensitized conditional knockouts exhibited lower total plasma OVA-specific IgG. Our results show that B cell-specific Plexin B2 plays a functional role in both early B cell development and GC B cell responses. Future work will evaluate the molecular mechanisms through which Plexin B2 regulates GC responses.

TH100. Macrophage-Conferred Innate Memory Augments Adaptive Immunity vs. MRSA Infection

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Staphylococcus aureus is the leading cause of skin and skin structure infection (SSSI), a primary portal of entry for invasive infection. Our prior studies discovered a role for protective innate memory against recurrent methicillin-resistant S. aureus (MRSA) SSSI. Using wild-type (WT) and *rag1*-deficient mice, we found prior infection (priming) protected from recurrent MRSA SSSI in the absence of adaptive immunity, indicating trained immunity. In the current study, correlates of protective memory were analyzed. In WT mice, cytokines correlated with protective memory in skin included increased IL-17, IL-6, MIG and RANTES. Cellular signatures of protection included increased Th17, M1 macrophage (Mf) and dendritic cell populations in abscesses, and total Mf in lymph nodes. Since Mf play important roles in innate memory, we investigated whether these cells can confer protective memory. Bone marrow derived Mf (BMDM) were generated from uninfected and infected mice and boosted *ex vivo* with heat-killed or live MRSA. Priming potentiated MRSA-specific phagocytic killing by WT BMDM *in vitro*, and their adoptive transfer into naïve skin afforded protective efficacy in vivo. However, adoptive transfer of primed BMDM from *rag1*$^{-/-}$ mice did not afford protection. Present findings indicate that protective immunity in recurrent MRSA infection involves specific innate memory conferred by Mf that augments the efficacy of adaptive immune mechanisms. These insights provide new targets for vaccine and immunotherapeutic development against MRSA.

TH118. Augmentation of Oral Immunotherapy with Tolerance-inducing Nanoparticles

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Roughly 15 million Americans suffer from food allergies which, in severe cases, can be life threatening. Allergic individuals typically manage their conditions through strict food avoidance and/or the

adminstration of antihistamine upon accidental exposure. Presently, oral immunotherapy (OIT) is the most efficacious option to achieve sustained unresponsiveness (SU) in allergic paitents but is limited by the risk of triggering anaphylaxis and requiring intensive medical supervision. Moreover, SU is only established in 13-36% of paitents undergoing the therapy. Several clinical studies indicate that SU after OIT is correlated with increased T regulatory cell populations, which suggests the supplementation of AIT with tolerogenic immunomodulatory factors will increase its efficacy. Polysaccharide A (PSA), a commensal molecule produced by the gut-symbiont *Bacterorides fragilis,* has been shown to have Treg-inducing capabilities within the gut. The tolerance-inducing capacity in combination with its polymeric structure makes PSA an intriguing biomaterial for the formulation of tolerogenic nanoparticles. We hypothesize that the encapsulation of allergen within PSA nanoparticles (NPs) will show significant improvements in efficacy and safety over traditional allergen-only oral immunotherapy due to PSA NPs ability to (i) induce Treg differentiation and deliver allergen simultaneously, and (ii) to shield the allergen from IgE receptor-mediated mast cell activation until internalization by intestinal dendritic cells. W show that PSA NPs can be readily fabricated using water/oil emulsification with glutaraldehyde crosslinking and maintain immunoregulatory capability, including TLR2 stimulation and $CD4^{+}IL\text{-}10^{+}$ T cell differentiation. PSA NPs have the potential to become a “plug-in-play” system to induce specific tolerance to any encapsulated allergen.

TH127. Local Production of IgE in the Colonic Mucosa of Food Allergic Patients

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Despite the fundamental role of IgE in allergy, the origin of human IgE is poorly understood. We analyzed juvenile polyps from patients with rectal bleeding and IgE sensitization and the mechanisms for IgE production. Patients were selected (n=105) and polyps were removed along with surrounding tissue (SCT). The inflammatory content of polyps (PT) was analyzed and nucleic acids were obtained from germinal centers (laser dissection microscopy) to study the class-switch recombination and VH gene (PCR and qPCR). Serum IgE and skin tests were assessed in all patients and cytokines were assessed (qPCR and ELISA). Polyps were found in 47.6% of patients, and 60 % showed a compatible history of allergy, with total and specific serum IgE positive in 90% of patients. We found higher frequencies of eosinophils in 86% of the polyps compared with SCT (43.06±3.16vs13.75±1.49 eosinophils per high-power field in JPvsSCT, $p< 0.001$) and IgE+ cells (88.00±15.95vs2.75±0.85 IgE+ cells PTvsSCT, $p< 0.0001$). PT contained higher levels of total IgE than SCT (56.83±18.57vs3.52±1.82)($p< 0.005$) and 40 % of the PT showed significantly increased levels of tissue milk-specific IgE. We found significantly higher levels of IL4, IL-5 and IL-13 in PT compared to SCT and active germinal centers with proliferating and AID-expressing B-cells. The analysis of RNAm from PT revealed local direct (εGLT and Iε-CμCT) and sequential (γ1GLT, γ3GLT, and Iγ-CεCT) class-switch recombination to IgE, and expression of VH1/7, VH3 and VH5 families. In conclusion, polyps showed a high content with local production of IgE in the colon of food-sensitized patients.

TH370. Therapeutic Efficacy of Curcumin in amelioration of Atopic Dermatitis in Mice

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Atopic dermatitis (AD) is a chronic skin inflammatory disease with high prevalence in children worldwide. Curcumin has gained great attention due to its anti-inflammatory and anti-allergic properties in a number of disease conditions. Recently, we have reported the beneficial potential of curcumin in prevention of OVA-induced AD in mice. The present study was undertaken to examine the efficacy of the phytochemical to exert therapeutic effect against AD. AD was induced in female Balb/c mice by epicutaneous application of OVA at the back skin site for 1 week followed by a resting period of two-weeks and same protocol was repeated thrice. Therapeutic potential of curcumin was examined by administration of the phytochemical either *via* intraperitoneal injection or through topical application. Systemic administration of curcumin resulted in amelioration of OVA-induced AD-like skin lesions. Further, the reduction in skin lesions was found to be associated with the decreased levels of Th2 cytokines (IL-4, IL-5 and IL-13) and restoration of redox balance in the skin. Remarkably, the topical application of curcumin seems to work with similar efficacy to blunt the expression of Th2 cytokines and restoration of skin histo-architecture as was observed when the drug was given through intraperitoneal route. Overall, our findings suggest that phytochemical based ointment(s) can offer newer treatment options for AD.

Autoimmunity

F66. Exhausted CD8 T Cell Subsets Determine Outcome in Teplizumab Treated Subjects At-Risk for T1D

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Teplizumab (FcR non-binding anti-CD3) treatment delayed onset of T1D in islet autoantibody positive at-risk subjects in a TrialNet trial. TIGIT+KLRG1+ CD8 T cells expanded in treated at-risk subjects. Here, we functionally define TIGIT+KLRG1+ CD8 subsets and link them to outcome in TN10. Consistent with CD8 T cell exhaustion, TIGIT+KLRG1+ cells express inhibitory receptors, produce inflammatory cytokines at lower levels than total memory cells, and transcriptionally resemble exhausted cells. While the total TIGIT+KLRG1+ CD8 T cell frequency is stable by flow cytometry over time in longitudinal analyses and in vitro cultures of untreated subjects, higher frequencies are negatively associated with CD127 expression (r=-0.61, p=0.0005) suggesting heterogeneity within TIGIT+KLRG1+ CD8 T cells. Heterogeneity in treated TN10 subjects was associated with outcome through Kaplan Meier analysis of flow cytometry clusters determined using FlowSOM on gated CD8 T cells. A common cluster resembled exhausted cells (EOMES+PD-1+KLRG1+TIGIT+), increased 2-fold upon treatment, and was associated with delay in T1D (p=0.0012). Smaller Ki67+ (< 1%) and CD127+

(12-18%) clusters also co-expressed EOMES and were associated with delay (p=0.003) and faster (p=0.00043) T1D onset, respectively. Suggestive of a reduction in activation and promotion of terminal exhaustion, treatment reduced the level of IFNγ secretion in CD127+TIGIT+KLRG1+ CD8 T cells from an average of 20% of cells prior to 8% immediately following treatment. Thus, our results support the interpretation that CD8 exhausted cells, a hypo-responsive cell type that is detrimental in cancer, instead, play a protective role in T1D and are expanded upon teplizumab treatment.

F78. Multiple Sclerosis risk allele in the HDAC7 locus impacts Treg function

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Multiple sclerosis (MS) is a highly heritable autoimmune disease of the central nervous system. The vast majority of MS risk loci, identified by genome wide association studies, are localized to gene enhancers active in stimulated T cells. These findings imply MS risk is mediated by relatively subtle changes to the epigenetic landscape of immune cells, which in turn affect the precise regulation of key genes. The latest association study has uncovered several low-frequency coding variants associated with MS risk, including a protective variant in HDAC7 (R166H). The gene codes for histone deacetylase 7, which mediates the removal of activating acetyl marks from histone tails to change enhancer states. We show HDAC7 regulates genes essential for the function of regulatory T cells (Treg), an immunomodulatory subset that is dysfunctional in MS patients. Tregs transfected with HDAC7 R166H constructs present with increased Treg function and suppression capacity compared to WT HDAC7 expressing Tregs. Transcriptomic analysis of HDAC7 R166H expressing Tregs reveal alterations in T helper and mitochondrial gene modules. Finally, conditional mono-allelic deletion of HDAC7 in Tregs increases severity of experimental autoimmune encephalitis, a mouse model of MS. Through the study of the HDAC7 R166H variant, we explore how changes to the epigenetic landscape can drive MS risk, using a known mediator of enhancer state. We demonstrate a risk allele impacts Treg function, implying a causal role for Treg dysfunction in MS. Finally, we highlight the importance of HDAC7 in the Treg compartment to maintain peripheral tolerance in the context of neuroinflammation.

F86. Developing Islet Antigen-Specific Chimeric Antigen Receptor for Regulatory T Cell Therapy in Type 1 Diabetes

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Early-phase clinical trial of *ex vivo* expanded polyclonal Tregs in T1D showed persistence of the infused Tregs and promising safety profile. However, efficacy in diabetes reversal will likely require islet antigen-specific Tregs that will be difficult to expand *ex vivo.* Chimeric antigen receptor (CAR) is one potential strategy to redirect polyclonal Tregs to islet antigens. We thus began constructing islet-specific CARs by screening a Fab library for clones that bind to 20 different candidate pancreatic antigens. Two clones have been validated to bind to human islets and one of which was for DPP6. For constructing anti-DPP6 CARs, we turned to DPP6-specific nanobodies derived from camelidae antibodies for their smaller size, more stable structure, low immunogenicity, and better access to target antigens. From a panel of DPP6 nanobodies, we selected one with consistent high reactivity to human primary islet cells to construct a CAR. The resulting DPP6-CAR is expressed at the cell surface in Jurkats and primary human $CD4^+$ T cells. When co-cultured with human islets, we observed a strong and specific activation of DPP6-CAR expressing T cells. Since the DPP6-CAR does not react with mouse islets, we have developed a humanized mouse model of autoimmune diabetes in which transplanted human islets are rejected by islet antigen-specific human T cells. Ongoing experiments are evaluating DPP6-CAR Tregs *in vitro* and *in vivo* toward the goal of determine the efficacy and safety of islet antigen-specific CAR Tregs. Results from this study will inform future efforts in translating this strategy to the clinic.

F143. Tolerogenic Tregitope in Factor V with superior inhibitory capacity as a potential immunotherapy in Autoimmunity and Allergy

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Tregitopes are regulatory T cell epitopes derived from conserved sequences in IgG that induce the body's natural regulatory T cells, and thereby suppress unwanted immune responses, maintain and generate antigen-specific tolerance, prevent the immune system from attacking self, maintaining homeostasis and self-tolerance avoiding the development of autoimmune diseases and allergies. Published work from our laboratory and others have shown that Tregitope-specific natural Tregs (nTregs) modulate T effector responses by inhibiting the activity of autoreactive effectors and/or by changing the phenotype of T effectors to adaptive aTregs in models of autoimmunity and allergy.

We have identified several Tregitopes in factor V (FV) using our *in-silico* tool that are promiscuous HLA-DR binders and have highly conserved TCR facing residues to other prevalent serum proteins, characteristic of Tregitopes. We have found that a novel factor V derived Tregitope (FVP4) significantly suppressed CD4 recall response to Tetanus Toxoid (TT) in an ex vivo assay using PBMCs from healthy donors. FVP4 co-culture with PBMC also reduced HLA DR expression on CD11c+ antigen-presenting cells. The inhibition of CD4 T effector cell activation and increase in the ratio of Treg/Teff cells is a direct

consequence of the activation of T regulatory cells and/or the conversion of TT-specific T effector to adaptive aTregs.

The delivery of this potent, newly identified FVP4 Tregitope will improve on existing Ag-specific therapies and restore adaptive tolerance by harnessing the power of natural T regulatory cell induction to selected disease antigens involved in organ-specific autoimmunity (such as T1D, Graves' disease) or environmental allergens.

F146. Methotrexate delays type 1 diabetes onset and inhibits diabetogenic immune responses in NOD mice

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Methotrexate (MTX) is effective in the treatment of autoimmune diseases such as rheumatoid arthritis and psoriasis. Type 1 diabetes (T1D) is an autoimmune disease, in which pancreatic islet β cells are mainly destroyed by diabetogenic CD8 T cells. The aim of the study was to investigate effects of MTX on T1D and diabetogenic immune responses in a non-obese diabetic (NOD) mouse model.

Female NOD mice were treated with low doses of MTX or PBS subcutaneously, diabetes development was monitored, and mouse tissues were assessed for insulitis, diabetogenic IGRP V7$^+$CD8$^+$ T cells and T regulatory cells (Tregs). Further, in vitro effects of MTX on 8.3 CD8 T cell proliferation and activation upon IGRP V7 peptide stimulation were also determined.

Diabetes onset was delayed in NOD mice after MTX treatment and islet insulitis scores were lower in MTX-treated mice than in PBS control mice. The proportion of circulating IGRP V7$^+$CD8$^+$ T cells in MTX-treated mice was reduced significantly. Adoptively-transferred 8.3 CD8 T cell proliferation in MTX-treated mice was largely abolished, whereas the fraction of FOXP3$^+$CD73$^+$ Tregs within the pancreatic lymph nodes was enhanced significantly after MTX treatment. Further, ex vivo experiments showed that MTX strongly inhibits IGRP V7-stimulated 8.3 CD8 T cell proliferation and expression of IFNγ and NKG2D upon IGRP V7 stimulation.

This study demonstrates that MTX treatment delays T1D development, inhibits insulitis and diabetogenic IGRP V7$^+$CD8$^+$ T cell proliferation, and increased the fraction of FOXP3$^+$CD73$^+$ Tregs in NOD mice. Thus, MTX may provide an effective and novel treatment for T1D.

F147. AIED Patients Demonstrate Unique Caspase-7 Mediated Processing of IL-1beta

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Interleukin-1β (IL-1β) is a key pro-inflammatory cytokine involved in the progression of many auto-inflammatory and autoimmune diseases including autoimmune inner ear disease (AIED). IL-1β inhibition has been shown to result in clinical hearing improvement in a small cohort of corticosteroid-resistant AIED patients. It is well accepted that IL-1β processing occurs through caspase-1 to generate a 17 kDa fragment of IL-1 β, however, a lesser-known caspase-mediated cleavage site may result in a 28 kDa fragment. We have identified that PBMCs from AIED patients uniquely generate this 28 kDa fragment in response to lipopolysaccharide (LPS). We synthesized and compared the biologic activity of the 28 kDa fragment to the 17 kDa IL-1β product treating PBMCs from AIED patients and control subjects with these peptide fragments. The 28 kDa fragment induces IL-6, TNF and CCL3 in PBMC. We sought to determine which caspase results in generation of 28 kDa of IL-1β. Interestingly, caspase-1 was not able to generate the observed 28 kDa fragment, however caspase-7 showed a dose and time dependent increase in 28 kDa band expression. Mass spectrometry confirmed that caspase-7 cleavage of pro-IL-1β generates the 28 kDa fragment observed in AIED patients. Collectively, *these* results provide an insight into a novel *mechanism* for IL-1β processing resulting in 28 kDa fragment generation and its proinflammatory effects. Work is underway to determine which component of PBMC directs the generation of 28kDa fragment.

F157. Transcriptomic Profiling of the Immune Compartment in the Tissue Environment of Psoriatic Arthritis

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Psoriasis is a chronic inflammatory skin disease affecting between 1.5-5% of the population in developed countries. Up to 30% of psoriasis patients also develop psoriatic arthritis (PsA) afflicting the joints. The aetiology of psoriasis and PsA is poorly understood. To address this gap in knowledge, we adopted a transcriptional approach to identify dysregulated immune networks in affected tissue.

Skin punch biopsies from lesional and morphologically normal skin of 4 PsA patients were enriched for CD45+ cells to obtain immune cells for subsequent RNA purification and RNAseq. Differently expressed genes (DEG) were identified and pathway analysis performed using the integrated Differential Expression and Pathway (iDEP) analysis tool.

We found that CD45+ cells from PsA lesional skin, compared to normal sites, were enhanced for DEG associated with immune processes (including *IL17A*, *FCN1*, and *CTLA4*), in particular, anti-microbial

responses (such as *DEF4BA* and *S100A8*) and immune cell chemotaxis (notably *CXCL13* and *SELPLG),* suggesting possible inflammatory responses to skin microbiota that may promote influx of circulating immune cells. Interestingly, lesional skin showed deficient expression of genes associated with tRNA metabolic processes (including aminoacyl tRNA synthetases e.g. *AARS*), suggesting protein translation defects that could contribute to immune cell dysfunction.

Our transcriptional approach provides a comprehensive overview of localised immunity in psoriasis and predicts intimate interactions with the peripheral immune system. Further studies are ongoing to uncover cell types involved, as well as parallels at other disease sites. These findings will facilitate the identification of novel targets for treatment of PsA.

F159. Deciphering the immune architecture of enthesitis-related arthritis

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Enthesitis-related arthritis (ERA), a common subtype of juvenile idiopathic arthritis (JIA) in Asia, carries a poor prognosis but limited knowledge on disease mechanism frustrates clinical diagnosis and treatment. We hypothesise multiple aberrations from the healthy immunome drive ERA pathogenesis, which demands a comprehensive high-dimensional interrogative strategy to assess the ERA immune architecture.

We examined peripheral blood mononuclear cells from 30 ERA patients within the first year of disease and 30 healthy paediatric controls with mass cytometry, using two extensive antibody panels encompassing key lineage and functional markers. Dimensional reduction and unsupervised clustering were performed to identify immune cell subsets differentially present in ERA patients. These subsets were statistically evaluated with reference to the healthy cohort and their association with disease activity determined.

We identified broad differences in the ERA circulatory immune architecture spanning both innate and adaptive immune cell populations, notably with the enrichment of naive CD4+ and CD8+ T cells alongside depletion of cytolytic NK cells ($CD56^{dim}CD16+$). In addition, the chemotactic and cytokine profiles of their subsets also differed in ERA patients, which is mechanistically relevant as this underscores their migratory capacities and potential effector roles in the ERA arthritic microenvironment.

This is the first study, via deep parameterisation afforded by mass cytometry, to demonstrate a concomitant dysregulation of both innate and adaptive immunity in ERA patients. Further mechanistic

studies of these immune cell subsets and their functional networks will inform development of prognostic markers of important clinical fates.

F163. High-Dimensional Immunophenotyping by Mass Cytometry Reveals Unique Cellular Alterations in Patients with Undiagnosed Inflammatory Diseases

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Few tools are available to evaluate immune dysregulation in patients with severe autoimmune or inflammatory conditions that do not conform to well-defined diseases. The ability to identify features of the aberrant immune response in these patients may help reveal underlying etiology and guide therapy. Here we used mass cytometry to study immune dysregulation in patients with immunologic conditions assessed in the Undiagnosed Diseases Network (UDN), a multi-center program evaluating rare disorders that defy diagnosis. We analyzed PBMCs from 16 UDN patients with inflammatory conditions plus 97 non-inflammatory controls, 25 lupus patients, and 20 rheumatoid arthritis patients as comparators using two 39-marker panels. We identified outliers as patients with an exceedingly high frequency of a specific cell subpopulation or expression of a marker, with a level elevated >2-fold above the next highest patient. This approach revealed outlier features in 4/16 UDN patients (25%) and 2/142 comparators (1.4%), indicating significant enrichment in UDN patients ($p < 0.001$). UDN cases included: $CD25^{hi}CD127^{-}$ Tregs comprising ~50% of $CD4^{+}$ T cells (average=4.7±4.1%) in a patient with erythroderma; Vδ2 γδ T cells comprising 27% of T cells (average=2.4±3.2%) in a patient with CNS disease; $GranzymeB^{+}/CD94^{+}$ T-cells comprising 35% of $CD4^{+}$ T cells (average=1.1±3.5%) in a patient with peripheral neuropathies; CD138 overexpression on monocytes/T cells (MFI=36; average=1.0±3.4) in a patient with CNS disease. Analyses also uncovered a patient with expanded $Bcl2^{+}/CD5^{+}$ B cells, subsequently diagnosed as leukemia. Thus, integrating high-dimensional cytometry with clinical and genetic findings offers a promising approach to identify pathogenic immune mechanisms in undiagnosed patients.

F166. Tetraspanins and miRNAs related to B cells isolated from urinary extracellular vesicles as biomarkers for lupus nephritis

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Lupus nephritis (LN) is a complication of systemic lupus erythematosus (SLE); it presents from asymptomatic mild proteinuria to glomerulonephritis that progresses rapidly to renal failure, affecting up to 60% of patients who have been diagnosed with SLE. Renal biopsy is useful to establish the specific class and severity of LN, but being an invasive procedure, it presents renal risks and complications such as macroscopic hematuria, peri-renal hematoma, infection, damage to adjacent organs and barely renal loss and death. It is currently known that in several diseases there is an increase in the number of extracellular vesicles (EVs) in different fluids, such as urine. Therefore, biomarkers can be obtained for the disease prognosis. Thus, in this work, the presence of urine EVs (uEVs) was evaluated in patients with active LN, SLE without renal activity, and in healthy individuals. Tetraspanins (CD37, CD53, tetraspanin33), and ADAM10 were identified in uEVs by flow cytometry and B-cell-related miRNAs by qPCR. We found a greater amount of uEVs, as well as tetraspanins and miRNAs in patients with active LN, than in healthy individuals or in patients with SLE without renal activity. In addition, some of these variables correlate with the clinical data of active disease, therefore, it is suggested that they can be used as biomarkers for the prognosis of LN.

F170. Compound heterozygosity for two NLRP3 mutations in a child with MOG-autoantibody positive encephalomyelitis

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Background:

Myelin Oligodendrocyte autoantibodies (antiMOG) occur in a subset of demyelinating neuroinflammatory disorders, termed antiMOG-associated encephalomyelitis (MOG-EM). NLRP3 GOF-mutations are associated with autoinflammatory conditions known as Cryopyrin Associated Periodic Fever syndromes. We present a case of MOG-EM with concomitant compound NLRP3 heterozygosity previously associated with autoinflammation.

Case:

A 2.5 year old boy presented with lethargy, behavioural changes and daily fevers for three weeks. Physical examination was noncontributory. Inflammatory markers were raised. An extensive infectious work-up was negative, imaging studies and bone marrow aspirate normal.

While daily fevers continued, he deteriorated neurologically, developing clonus, hyperreflexia and encephalopathy and requiring admission to intensive care. MRI brain/spine demonstrated acute

disseminating encephalomyelitis affecting deep grey matter, and transverse myelitis. Repeat lumbar puncture was nonreactive but antiMOG positive, constituting a diagnosis of antiMOG-positive encephalomyelitis and longitudinally extensive transverse myelitis (antiMOG-EM/LETM). Following steroid pulse and IVIG, he made a gradual but complete recovery. An autoinflammatory gene panel identified compound heterozygosity for two NLRP3 missense mutations, one of which previously associated with autoinflammation, one novel. The patient remains under close clinical surveillance. IL1-blockade has not been commenced.

Discussion:

We present a patient with antiMOG-EM/LETM, whose genotype places him onto the autoinflammatory spectrum. This case may illustrate a role for inflammasomopathies in antiMOG-spectrum and similar disorders not previously associated with the inflammasome. A larger-scale study investigating the role of NLRP3 in neuroinflammatory disorders may provide important pathophysiological insights and potentially novel avenues for treatment.

F184. The Role of Aire in the selection of Regulatory T cells in Diabetes

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In autoimmune diseases like T1D, it is proposed that failure in central tolerance mechanisms towards pancreatic islet self-antigens leads to the escape of pathogenic clones and loss of critical immunosuppressive antigen-specific Tregs. The importance of Aire in maintaining central tolerance against self-reactive antigens is evidenced by the multiorgan autoimmune disease seen in individuals with mutation the Aire gene. When Aire-expression is specifically ablated in mouse mTECs, there is a dramatic increase in the number of self-reactive T cells that escape negative selection, in addition, a dramatic loss of FoxP3$^+$ Tregs. Utilizing novel high-throughput platforms and single-cell DNA-barcoding technology has allowed us to assess several thousand CD4$^+$ T-cell clones. The use of FoxP3-GFP reporter mice permits us to identify individual FoxP3$^+$Tregs that have undergone Aire-dependent selection in the thymus and compare the TCR repertoire to mice that have had Aire specifically ablated from the mTECs. Identification of unique clones and gene signatures within our large datasets has allowed us to qualitatively assess the functional role of the identified TCRs within the model of T1D. We have begun comparing our extensive datasets of insulin-specific-TCRs and TCRs that we have previously identified from diabetic animals to our Aire-dependent-TCRs. In addition, the peptide specificities of the identified TCRs are currently being evaluated using *Baculovirus* MHC-II peptide-libraries. Our proposed study aims to expand our knowledge on TCR repertoire of Aire-dependent FoxP3$^+$Tregs and the antigen-specificity in T1D, improving our understanding of the respective function

of thymic-derived antigen-specific Treg suppression in peripheral tissues associated with autoimmune T1D.

F185. The Killer Immunoglobulin-like Receptor KIR3DL1 in Combination with HLA-Bw4 is Associated with Pediatric Acute-onset Neuropsychiatric Syndrome (PANS)

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Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) is characterized by abrupt onset of obsessive-compulsive disorder (OCD) and/or food restriction with other specified neuropsychiatric symptoms. PANS patients have been observed to have co-existing arthritis, immunodeficiency and other autoimmune diseases, and/or first degree family members with autoimmune diseases. Our previous study of HLA variation in PANS patients versus respective healthy controls of European ancestry showed significant HLA-B associations including HLA-B38, HLA-B52 and HLA-B27 allotypes (being predominantly for HLA-B*27:02 allele), having in common that they present the Bw4 serological epitope. Since Bw4 is the ligand of the inhibitory receptor KIR3DL1 on the surface of natural killer cells, we extended our work to characterize polymorphism of KIR3DL1 in a cohort of PANS patients of European ancestry (n=134) and respective ethnically-matched healthy controls (n=1,748). We performed a probe capture enrichment NGS-based KIR genotyping method with a custom bioinformatics pipeline (Norman et al., AJHG 2016) to determine polymorphism of KIR3DL1, and we analyzed the KIR3DL1 genotyping data in the context of its ligand Bw4 (encoded by certain HLA-A and HLA-B allotypes, where this NGS-based HLA data was pre-existing). We found that KIR3DL1*002 allotype in the presence of Bw4 was strongly associated with PANS patients when compared to healthy controls (OR=1.84, 95% CI, 1.18-2.78, p=0.005). Interestingly, KIR3DL1*002 encodes a KIR inhibitory allotype that is expressed at high levels on the surface of NK cells (Saunders et al., JEM 2016).Thus, we suggest this KIR-HLA interaction may be involved on the immune dysregulation events being part of the pathophysiology of PANS.

F186. Single cell transcriptome analysis of circulating plasmacytoid dendritic cells and switched memory B-cells in SLE patients reveals differential responsiveness to Type I IFN

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Background/Purpose: Both plasmacytoid dendritic cells (pDCs) and switched memory B cells (SMBCs) are key effector cells in systemic lupus erythematosus. We performed single-cell RNA sequencing in pDCs and SMBCs from SLE patients and controls to assess cellular sub-groups within these lineages.

Methods: pDCs and SMBCs from SLE patients (n=10) and Healthy controls (HC; n=5) were purified by magnetic separation. Single cells were captured and visually confirmed as single cells using the Fluidigm C1 HT system. pDCs and SMBCs were clustered using UMAP and pseudo-temporal analyses. Additionally, bulk RNA sequencing of in vitro IFN-α and β stimulated pDCs and SMBCs from HC was performed to define cell type specific IFN-α and β response modules.

Results: In pDCs, type I IFN response was the major determinant of overall clustering patterns. IFN signature in pDCs correlated with circulating type I IFN. In SMBCs, the overall clustering pattern was independent of IFN signature. SMBC clusters were defined by cellular activation and proliferation genes (*HLA-DRs* and *CREB1)*, and nucleic acid processing genes (*DNASE1* and *SNORD3B-1)*. When examined IFN-specific signatures, pDCs were more IFN-β primed, while IFN-α signature predominated in SMBCs. Of 1773 SLE-pDCs, zero expressed IFN-α or IFN-β transcripts, suggesting that IFN-secreting pDCs are very infrequent in circulation.

Conclusions: Type I IFN induced transcripts are important to pDC diversity, while in SMBCs transcripts related to cellular activation and nucleic acid processing are critical markers of transcriptional heterogeneity. The data also suggest that pDCs and SMBCs differ in their responsiveness to IFN-α vs. IFN-β in SLE.

F188. ILT7 and BDCA2 Regulation of Plasmacytoid Cells in Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease in which plasmacytoid dendritic cells (PDCs) are pathologically activated. In SLE, PDCs activated through toll like receptor 7/9 produce interferons, inflammatory cytokines and become potent antigen presenting cells. PDC activation can be inhibited by ligation of the regulatory surface receptors, immunoglobulin like transcript 7 (ILT7) and blood dendritic cell antigen 4 (BDCA4).

Objective: To measure expression levels and function of ILT7 and BDCA2 using PDCs from lupus patients and controls.

Methods: ILT7 and BDCA2 levels on PDCs from 50 lupus patients and 20 controls were measured by quantitative flow cytometry. ILT7 and BDCA2 function was assessed by crosslinking ILT7 and BDCA2 on TLR9 activated PDCs and measuring IFN-α and TNF-α in 18-hour supernatants. Data were analyzed for correlations with autoantibody titers, IFN serum levels, and disease symptoms and severity.

Results: ILT7 and BDCA2 expression levels were significantly decreased in patients with high disease activity (SLEDAI) scores. ILT7 and BDCA2 levels inversely correlated with serum ANA and dsDNA titers. ILT7 and BDCA2 levels were 50% lower in patients with high autoantibody titers compared to controls. Preliminary studies indicate the receptor function in these patients is dysregulated.

Conclusions: PDCs appear to be dysregulated in patients with severe disease and could be a contributing factor to disease progression in these patients. Low levels and decreased function of ILT7 and BDCA2 in patients with high SLEDAI scores suggests that these patients may respond poorly to therapies currently in clinical trials that target these receptors.

F193. Anti-CD45RC mAbs Immunotherapy Controls the Development of Auto-Immune Symptoms in an APECED Rat Model of AIRE Deficiency

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Auto-immune regulator (AIRE) is a key transcription regulator that allows negative selection by promoting the expression of tissue restricted antigens in the thymus. In human, AIRE-deficiency results in the development of autoimmune-polyendocrinopathy-candidiasis-ectodermal-dystrophy (APECED), a lethal autoimmune disease characterized by lesions of multiple peripheral organs and production of many autoantibodies. To date, no cure is available.

Recently, our team generated the first *Aire*$^{-/-}$ rat model. In contrast to mouse models, these animals harbor several key features of APECED and are therefore a pertinent model for preclinical studies. We previously showed that targeting CD45RC enables a selective depletion of effector T cells (Teffs) while preserving and boosting regulatory T cells (Tregs). Moreover, anti-CD45RC mAb therapy was

protective in transplantation. To address the potential of anti-CD45RC immunotherapy to control APECED autoimmune symptoms, we tested this treatment in our model.

First, we demonstrated that anti-CD45RC mAbs efficiently reduced alopecia and vitiligo presented by *Aire*$^{-/-}$ animals. Besides, isotype-treated *Aire*$^{-/-}$ rats showed complete destruction of exocrine pancreas and loss of thymus structure whereas these organs were preserved by anti-CD45RC mAbs therapy. Interestingly, this treatment also decreased the production of autoantibodies and modified Tregs' transcriptome. Finally, analysis of PBMCs from APECED patients confirmed that the expression of CD45RC was similar to the one observed in *Aire*$^{-/-}$ rats.

In conclusion, we demonstrated that anti-CD45RC immunotherapy was able to control the development of auto-immune symptoms in *Aire*$^{-/-}$ rats. Furthermore, CD45RC expression was conserved between our model and APECED patients underlying the clinical potential of CD45RC targeting in this disease.

F196. Genes Implicated as Causative in Primary Immunodeficiency are Up-Regulated in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a polygenic autoimmune disease defined by hyper-reactivity of the immune system. In contrast, persons with primary immunodeficiency (PID) have specific defects in molecular pathways required for complete immune responsiveness. To determine whether PID-defined molecular pathways are involved in the pathogenesis of autoimmunity, we asked whether causal PID genes are abnormally expressed in SLE. A novel, comprehensive database of 450 PID genes was created and curated by extensive primary literature mining. PID genes were clustered based on protein-protein interactions and immunological functions of the gene clusters determined. Protein-protein interaction clustering of PID genes formed 16 distinct groups with significant intracluster and intercluster connectivity, predominantly categorized by DNA repair, immune cell surface markers, pattern recognition receptors, secreted immune proteins, and pro-proliferation genes. Of the 450 PID genes, 400 were differentially expressed in SLE patients, with overexpression associated with increased disease activity determined by the SLE Disease Activity Index. Applying a combination of a Variable Autoencoder to sort a cohort of 1620 SLE patients based on clinical features and machine learning to classify subjects based on gene expression, we documented that up-regulation of PID genes was associated with SLE disease activity. Together, these data represent a large scale, comprehensive analysis of the role of PID genes in SLE, and demonstrated that genes encoding immune checkpoints are over-expressed in SLE patients and can be used to classify immunologic activity in lupus.

F200. LAG3 limits diabetogenicity of intra-islet CD8 T cells.

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Control of effector T cells is necessary to limit autoimmune diabetes (AD). One mechanism by which this is achieved is through inhibitory receptor (IR) expression. Lymphocyte Activating Gene 3 (LAG3) is one critical IR that limits disease progression and incidence of AD. Using a mouse model in which LAG3

deletion is restricted to CD8$^+$ T cells, *Lag3*$^{L/L\text{-}YFP}$E8i$^{CRE\text{-}GFP}$.NOD, and wild type (WT) controls, E8i$^{CRE\text{-}GFP}$.NOD, **we find that CD8$^+$ T cell-restricted deletion of LAG3 is sufficient to accelerate diabetes incidence and insulitis**. Interestingly, we see *Lag3*$^{L/L\text{-}YFP}$E8i$^{CRE\text{-}GFP}$.NOD mice have an increased intra-islet CD8:Treg ratio, and increased tetramer (Nrpv7, IGRP mimotope) staining. **These observations drive our hypothesis that LAG3 limits those autoreactive CD8$^+$ T cell.** 5' single cell RNAseq reveals that LAG3-deficient (*Lag3*$^{-/-}$) intra-islet CD8$^+$ T cells are transcriptionally unique compared to WT controls, exhibiting a more activated phenotype along with lower geneset enrichment for markers of exhaustion. This was validated by flow cytometry in which WT CD8$^+$ T cells possess an expanded PD1^{Hi}, Tox$^+$ population, while *Lag3*$^{-/-}$ CD8$^+$ T cells have an expanded PD1^{mid} population, express more CD44, KLRG1, BrdU, Ki67, and less aCasp3. This indicates that WT CD8$^+$ T cells are able to appropriately upregulate PD1 and Tox in response to chronic self-antigen stimulation, while *Lag3*$^{-/-}$ CD8$^+$ T cells maintain an activated population. **Strikingly, LAG3 deletion on CD8$^+$ T cells appears to be sufficient to overcome a normal "exhaustion-like" program in intra-islet CD8$^+$ T cells, resulting in accelerated diabetes incidence and highlighting an important for LAG3 and exhaustion programs in AD.**

F201. Anti-inflammatory effect of Dimethyl Fumarate in the Intestinal Mucosa of Mice with Experimental Autoimmune Encephalomyelitis

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Dimethyl fumarate (DMF; Tecfidera, Biogen) is an oral medication utilized to treat Relapsing-Remitting forms of Multiple Sclerosis (MS). DMF treatment is associated with reduced disease activity in MS patients. However, the mechanisms by which this compound functions are not completely understood. This work aimed to investigate the effect of DMF administration in the intestinal mucosa of mice with Experimental Autoimmune Encephalomyelitis (EAE), the animal model to study the immunological response in Multiple Sclerosis (MS). C57BL/6 female mice were immunized to induce EAE. DMF or its vehicle were administered by gavage twice a day, starting on day 3 post immunization, in the concentrations 7.5 and 30 mg/kg. Mesenteric lymph nodes (mLN) were analysed by flow cytometry and Real-Time Polymerase Chain Reaction (RT-PCR). The analysis of T cells by flow cytometry showed no difference in the CD4+Foxp3+ regulatory T cells population after 10 and 15 days of treatment. However, a higher total expression of Foxp3 mRNA was observed on mLN with 10 and 15 days of DMF administration. The RT-PCR also showed higher levels of anti-inflammatory cytokines, such as IL-10, TGFb and IL-27, in the animals that received DMF. Altogether, our data show the production of anti-inflammatory molecules in the gut, which might contribute to DMF beneficial effects. These findings indicate that the gut could be an alternative site of action of DMF, which may contribute to its effects of reducing disease severity MS patients.

F202. Dimethyl Fumarate Induces Apoptosis of Activated Lymphocytes. Could Apoptosis Be Related to Lymphopenia in Multiple Sclerosis Patients?

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Dimethyl fumarate (DMF, Tecfidera, Biogen) is an ester of fumaric acid that is used as an oral medication for treatment of Multiple Sclerosis (MS). This drug has shown positive effects reducing clinical disease activity in relapsing-remitting MS (RRMS). DMF is hydrolyzed to monomethyl fumarate (MMF), which is considered its active metabolite, by intestinal esterases. A relevant complication that might appear with DMF treatment is lymphopenia. Our study aimed to evaluate if the lymphopenia observed is a result of the T cells entering on the apoptosis pathway. We used murine cells and human peripheral blood mononuclear cells (PBMC) in culture with DMF and MMF in different concentrations. Firstly, the cells were stimulated with anti-CD3 and anti-CD28 antibodies. After that, DMF, MMF or control were added to the cell culture and incubated for 24 hours. To access apoptosis rate, we measured the expression of the phosphatidylserine (PS). PS is naturally encountered on the inner part of the cell membrane, but it migrates to the outside layer when apoptosis begins. We used a labelled Annexin V, which is a natural binder for PS, to evaluate the apoptosis rate by flow cytometry. Moreover, stimulated T cells from both murine cells and human PBMC showed an increase on the apoptosis when they were treated with DMF and MMF in vitro. Our data indicates that the lymphopenia observed in MS might be related to an increase on T cell apoptosis.

Financial support: FAPESP, CNPq, CAPES

F203. Determination of cytokines and Indoleamine 2,3 dioxygenase in peripheral blood of Multiple sclerosis patients with depression/anxiety symptoms treated with IFNβ and Fingolimod

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Depressive and anxious disorder occur in up to 50% of people living with MS, which represents 2-3 times higher than those of the general population. Numerous etiological factors may contribute to the depression in MS patients including lesion burden, inflammatory response, as well as stressors that accompany living with an unpredictable disabling disease. Recently, an extensive body of data demonstrates that depression is associated with inflammatory response. Proinflammatory cytokines induce classical depression symptoms. Activation of inflammatory response also induces the production of indoleamine 2, 3 dioxygenase (IDO). IDO catabolizes tryptophan, the amino acid precursor of serotonin and melatonin, which may contribute with depression symptoms. In this study, we quantified IDO and pro and anti-inflammatory cytokines in two groups of patients with MS and depression treated with IFNβ and fingolimod. The results showed that patients treated with IFNβ had increased IDO expression while inflammatory cytokines were decreased. On the other hand, patients with MS and

depressive symptoms treated with fingolimod had significantly decreased IDO expression and significantly increased cytokines proinflammatory such as TNFα and IL-6, in levels comparable to patients with depression only. Our data indicate that the different drugs used to treat MS also have different mechanisms of maintaining depressive symptoms in patients with MS, which requires careful clinical follow-up of these patients.

Financial support: FAPESP, CNPq, CAPES

F204. GFAP distinguishes active AQP4-NMOSD from other inflammatory spinal cord lesions but does not correlate with MRI lesion load

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Neuromyelitis optica spectrum disorders (NMOSD) are inflammatory diseases of the central nervous system characterized by extensive long spinal cord lesions (LSCL). 60-90 % of NMOSD are positive for antibody recognizing aquaporin-4 (AQP4) expressed at the foot processes of astrocyte (AQP4-NMOSD). Recently, among anti-AQP4 antibody-negative NMOSD, 7.4-39 % were positive for anti-myelin oligodendrocyte glycoprotein (MOG) antibody (MOG-NMOSD). In the past, astrocytic marker such as SB100 and glial fibrillary acidic protein (GFAP) has been investigated as diagnostic biomarkers for AQP4-NMOSD. However, the possibility that GFAP merely reflects the extent of spinal cord damage has not been fully investigated. In this study, we retrospectively measured GFAP in frozen cerebral spinal cord fluid samples obtained before acute phase treatment in active inflammatory spinal cord lesions including AQP4-NMOSD. GFAP in AQP4-NMOSD (19 patients, mean 236.5 μg/ml) was statistically higher than in MOG-NMOSD (5 patients, mean 0.41 μg/ml, U-test, $p=1.82\times10^{-3}$) and in relapsing remitting multiple sclerosis (16 patients, mean 0.79 μg/ml, U-test, $p=1.46\times10^{-5}$). GFAP elevation was observed regardless of prior oral steroid intake and its sharp drop was confirmed after administration of intravenous methylprednisolone in concordance with neurological recovery. Correlation between GFAP and spinal MRI T2-lesion load was not found in our study. Over all, we propose GFAP as a biomarker in distinguishing active astrocytic damage in AQP4-NMOSD spinal cord lesion from other inflammatory myelitis. Its elevation is not dependent on the extent of the damage and may be a sensitive surrogated marker for determining the presence of acute inflammation in AQP4-NMOSD.

F208. Extracellular ADP/P2Y1 axis mediates neutrophil chemotaxis in inflammatory arthritis

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Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily affects the joints and associated with excessive immune cell infiltration. However, the cross-talk between tissue-resident and infiltrated immune cells in RA development remains unknown. Here, we demonstrated synovial macrophages exacerbate neutrophil-driven joint damage in RA through ADP/$P2Y_1$ signaling. We showed that extracellular ADP (eADP) and its receptors are all increased obviously in joints of RA model mice, and eADP enhances neutrophil infiltration through macrophages produced CXCL-2. Accordingly, the arthritis mouse model had more neutrophils in inflamed joints through ADP injection, whereas $P2Y_1$ deficiency and pharmacologic inhibition restored arthritis severity to baseline levels suggested the dominant role of ADP/$P2Y_1$ signaling in RA formation. Moreover, the cellular activity of ADP/$P2Y_1$-mediated CXCL-2 production was dependent on Ca^{2+}/$G_{\alpha q}$-NF-κB/NFAT pathway in macrophages. Overall, this study reveals a nonredundant role of eADP as a trigger in the pathogenesis of RA through neutrophil recruitment and disrupting tissue homeostasis and function.

F211. Lymphoma driver mutations in the pathogenic evolution of an iconic human autoantibody

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Despite affecting up to 10% of people, the pathogenesis of human autoimmune diseases remains poorly understood. A major hurdle in deciphering the underlying pathogenesis is distinguishing pathogenic lymphocytes that drive disease in amongst normal lymphocytes that contribute to host defence and studying their genomic and proteomic characteristics with the required precision. Recent advances in single cell genomics provides a powerful way to identify and characterize “rogue” lymphocytes within heterogenous immune populations in patients with autoimmune disease.

Here we apply single cell multi-omic technologies to measure transcriptomic, proteomic, genomic and epigenetic information from rare circulating B lymphocytes making pathogenic rheumatoid factor autoantibodies in multiples cases of cryoglobulinemic purpura with primary Sjogren’s syndrome. These rogue B cells making pathogenic autoantibodies were found to comprise clonal trees accumulating mutations in their B-cell receptors which conferred pathogenicity by causing the antigen-bound autoantibodies to undergo phase transition to insoluble aggregates at lower temperatures. When

compared to the patient's normal memory B cells, the rogue B cell clones were found to express an aberrant gene expression profile and displayed genome-wide hypomethylation. Lymphoma driver mutations in genes regulating B cell proliferation and activation – *CARD11*, *TNFAPI3*, *CCND3*, *BTG2* and *KLHL6* – were present in the rogue B cells producing the pathogenic autoantibody, providing a novel explanation for how these cells escaped immune tolerance checkpoints.

These results demonstrate that single-cell multi-omic technologies can be used to characterise pathogenic lymphocytes in autoimmune disease and provide direct evidence for a shared pathogenesis of autoimmunity and lymphoid malignancy.

F212. Ion channel-independent role of postsynaptic NMDA receptors in contralateral homotopic cortical innervation

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Anti-NMDAR encephalitis is an autoimmune disease associated with antibodies against NMDAR, with a predilection for children and young adults. Patients present with psychosis, seizures and abnormal movements. Abnormal movements are seen in around 90% cases, and are clinically prominent in children, indicating that motor-sensory circuits may be disrupted in these patients. The callosal connections in primary somatosensory cortex (S1) are especially critical for processing afferent somatosensory inputs and the integration of sensory and motor signals necessary for skilled movement. The influences controlling the callosal innervation pattern in S1 are not clear. We found that deleting NMDAR from the cortex disrupts callosal projections to the primary/secondary somatosensory cortex (S1/S2) border and leads to axons being uniformly distributed throughout S1. By combining genetic and pharmacological manipulations, commercial antibody- and patient derived monoclonal autoantibody-mediated loss of function, we found that loss of NR2B-containing NMDAR specifically in target S1 is the cause of this phenotype. Furthermore, we found that NMDAR function in callosal circuit formation is independent of ion channel function but dependent on interactions with the EphrinB/EphB signaling pathway. Thus, NMDAR in target S1 determines the formation of callosal somatosensory circuits. This work shows for the first time a molecular mechanism by which NMDAR regulate the anatomic formation of a circuit in the developing brain by affecting axon guidance. Moreover, because anti-NMDAR autoantibodies in pediatric patients cause persistent behavior deficits, our findings have significant pathophysiologic and therapeutic implications.

F213. The effects of germline STAT3-activating mutations from autoimmunity and lymphoid malignancy on mouse and human T cells

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Signal transducer and activator of transcription 3 (STAT3) regulates gene expression downstream of cell surface cytokine and hormone receptors. Heterozygous germline loss of function *STAT3* mutations lead to the primary immunodeficiency hyper-IgE syndrome while somatic activating *STAT3* mutations are highly recurrent in human solid organ and immune malignancies. Germline heterozygous activating *STAT3* mutations result in early-onset autoimmune disease, with aspects of immunodeficiency. Affected individuals share characteristics with autoimmune lymphoproliferative syndrome and immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, including reduced T regulatory cell numbers. They present with variable early-onset autoimmune symptoms including type 1 diabetes, rheumatoid arthritis, gut enteropathies and autoimmune cytopenias. Whilst many effects of STAT3 loss-of-function on immune cells have been described, the mechanisms behind autoimmunity and immunodeficiency in patients with activating *STAT3* mutations remain unclear.

Here, we present a detailed characterisation of T cell development and maturation in young and old mice on two different backgrounds with Crispr-engineered germline activating mutations in two different domains of STAT3. We use mixed bone marrow chimeras, flow cytometry, T cell receptor deep sequencing and high throughput single-cell transcriptomics to reveal cell-extrinsic and -intrinsic roles of overactive STAT3 in T cells in autoimmunity and immune malignancy. These mice provide a model to test the effect of various therapeutic agents *in vivo*. To our knowledge, this is the first report of mice with *Stat3* germline activating mutations identical to those in autoimmunity or malignancy. We validate our key findings in humans with gain of function germline *STAT3* mutations and childhood-onset autoimmunity.

F216. High-throughput Single-cell Sequencing Identifies a Subset of Arthritogenic T Cells Enriched in Nr4a1 and is Associated with Markers of Anergy and Exhaustion

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We developed a model to identify and study antigen-specific T cell responses in arthritis using a specific marker of T cell receptor (TCR) signaling—Nur77 (NR4A1)—and identified antigen-activated T cells in the SKG arthritis model and in patients with rheumatoid arthritis (RA). Using a fluorescent reporter of Nur77 expression in SKG mice, we found that higher levels of Nur77-eGFP in SKG CD4 T cells identified a subset enriched for highly arthritogenic and autoreactive T cells. The enhanced autoreactivity was associated with increased sensitivity to interleukin-6 (IL-6) receptor signaling and ability to more readily differentiate into IL-17–producing cells, likely contributing to their arthritogenicity. Single-cell RNA-Sequencing paired with TCR-Sequencing of naïve SKG and control CD4 T cells sorted based on their Nur77-eGFP levels reveals that naïve SKG CD4 T cells are associated with transcriptional markers of IL-6 signaling and Th17 differentiation prior to arthritis development. Further transcriptional analysis of these cells by leiden clustering identifies a subset marked by high *Nr4a1* expression and reveals heterogeneity in this cluster, likely reflecting cells that have experienced chronic from acute TCR stimulation. This *Nr4a1*-high cluster is associated with markers of anergy and exhaustion, which may be an adaptive response to chronic antigen-signaling. These findings are currently being examined in our human RA synovial T cell transcriptomic dataset. These data highlight

a functional correlate between *Nr4a1* expression and Nur77 levels in a highly arthritogenic T cell population with heightened IL-6 sensitivity in SKG mice. These findings may have translatable implications for human RA.

F237. Low-dose interleukin 2 in children with recently diagnosed type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled, dose-finding study

Michelle Rosenzwajg[1], Randa Salet[2], Roberta Lorenzon[3], Nicolas Tchitchek[4], Alexandra Roux[3], Claude Bernard[3], Jean Claude Carel[5], Caroline Storey[5], Michel Polak[6], Jacques Beltrand[6], Chloé Amouyal[3], Agnès Hartemann[3], Pierre Corbeau[7], Eric Vicaut[8], Cecile Bibal[9], Pierre Bougnères[9], Tu-Anh Tran[10] and David Klatzmann[1]

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Background: Low-dose interleukin 2 (ld-IL2) selectively activates and expands regulatory T cells (Tregs) and has the potential to skew the regulatory/effector T cell balance towards improved regulation. We performed a dose-finding clinical trial in children with type 1 diabetes (T1D) **(**NCT01862120).

Methods: DF-IL2-Child was a multicentre, double-blinded, placebo-controlled**,** phase 1/2 trial: 24 children with recently diagnosed T1D were randomised in placebo or IL-2 at doses of 0.125, 0.250, or 0.500 MIU/m^2, given daily for a 5-day and then fortnightly for 1 year.

Results: There were no serious adverse events. Non-serious adverse events were transient and mild to moderate. Ld-IL2 induced a dose-dependent increase in the percentage of Tregs. However, the individual Treg responses to IL-2 were variable. Seven patients, all among those treated with the 0.250 and 0.500 MIU/m^2/day doses, were Treg high-responders. At baseline, they had lower Treg proportions than Treg low-responders, and serum sIL-2RA and VEGFR2 predicted the Treg response. There was no significant change in glycaemic control in any of the dose groups but there was an improved maintenance of induced C-peptide production at one year in the 7 Treg high-responders

Conclusion: The safety profile at all doses, the dose-dependent effects on Tregs and the observed variability of the Treg response to ld-IL2 in newly diagnosed T1D children call for use of the highest dose in future developments. The better preservation of insulin production in Treg high-responders supports the potential of Tregs in regulating autoimmunity in T1D and warrants pursuing the investigation of ld-IL2 for its treatment.

F238. Low-dose interleukin-2 selectively expand and activate regulatory T cells across 13 autoimmune diseases.

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Background Regulatory T cells (Tregs) prevent autoimmunity and control inflammation. As low-dose interleukin-2 (ld-IL2) expands and activates Tregs, it has a broad therapeutic potential for any autoimmune or inflammatory disease (AIID). We performed a disease-finding "basket trial" (TRANSREG NCT01988506) in patients affected by one of 11 different AIID and reported the outcome of the first 46 patients (*Rosenzwajg et al, ARD 2019*). Here, we report the results from 78 patients with one of 13 different AIID.

Methods We performed a prospective, open label, phase I-IIa study in 78 patients with a mild to moderate form of one of 13 selected AIID. All patients received ld-IL2 (1 million IU/day) for 5 days, followed by fortnightly injections for 6 months. Deep immunophenotyping was performed before and after 5 days of ld-IL2.

Results ld-IL2 significantly expands both memory Tregs as well as naïve Tregs, including recent thymic emigrant Tregs. It also activates Tregs as demonstrated by the significantly increased expression of HLA-DR, CD39, CD73, GITR, CTLA-4. Similar results were observed across the different AIID.

Conclusion ld-IL2 "universally" improves Treg fitness across 13 autoimmune and inflammatory disease.

F239. Discovery and Characterization of ANB032, a Novel BTLA/HVEM Checkpoint Modulator for Autoimmune/Inflammatory Disease

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Human genetics and animal studies demonstrate a role for B and T lymphocyte attenuator (BTLA) in autoimmune disease. BTLA is an immune checkpoint molecule that contributes to the regulation of T cell, B cell and dendritic cell function through its interaction with its ligand herpesvirus entry mediator (HVEM) on cells in trans, and on the same cell in cis. Trans BTLA/HVEM interaction results in inflammatory costimulatory signaling through HVEM, while cis BTLA/HVEM interaction prevents costimulatory signaling of HVEM by numerous HVEM ligands, resulting in broad suppression of HVEM ligand-mediated immune cell activity.

We have discovered a novel anti-BTLA monoclonal antibody, ANB032, that enhances the BTLA/HVEM cis complex formation and does not antagonize the trans interaction. Hydrogen-deuterium exchange mapping of the ANB032 binding epitope on BTLA demonstrated that ANB032 bound a region distal from the HVEM binding site, providing evidence to support the lack of disruption of the BTLA/HVEM complex by ANB032. In cell-based assays, ANB032 functionally inhibited BTLA/HVEM trans costimulation and promoted cis BTLA/HVEM suppression of HVEM signaling.

In vivo, ANB032 was highly efficacious in a dose dependent manner when tested in a human PBMC-NOD-*scid IL2rγ^{null}* (NSG) graft versus host disease model, while an anti-BTLA antibody that antagonized the BTLA/HVEM interaction had no therapeutic effect. Thus, the ability of ANB032 to stabilize the cis BTLA/HVEM interaction and prevent inflammatory HVEM costimulation may lead to reduction of pathogenesis in human autoimmune/inflammatory diseases.

F241. Autoreactive B cells escape peripheral checkpoint in ANCA-associated vasculitis and Sjögren's Syndrome

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B cells play a central role in many autoimmune diseases (AIDs) including ANCA-associated vasculitis (AAV) and primary Sjögren's syndrome (pSS), but little is known on the characteristics of autoreactive B cells in humans. This study aims at characterizing circulating autoantigen (PR3 ou SSA)-specific B-cells in patients with AAV and pSS compared to healthy subjects.

We developed a new flow-cytometry method to detect circulating auto-reactive B cell based on the specificity of their B-cell receptor (BCR). Phenotype analysis showed that antigen-specific B cells in patients have a memory phenotype compared with healthy controls (**5 to 9% are IgG-expressing memory B cells**). It suggests that in AID, theses auto-reactive cells are able to differentiate into IgG isotype-switched cells and escape peripheral tolerance checkpoint but not in healthy subjects. Interestingly, Natural auto-reactive B cells present in healthy subjects, are able to secrete only IgM isotype autoantibodies upon *in vitro* stimulation but not IgG class switched antibodies. A genomic analysis of the antibody repertoire as well as a transcriptional profiling of these cells by single-cell RNA seq is ongoing to understand further the differences of these autoreactive B cells between healthy subjects and patients with AIDs.

Our results suggest that autoreactive B cells escape peripheral tolerance checkpoint and are able to differentiate into IgG isotype-switched cells in patients with AIDs but not in healthy subjects.

F242. Autoantibody Reversion: Changing Risk Categories in Multiple-Autoantibody Positive Individuals

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Objective

Nearly all individuals with multiple (≥2) islet autoantibodies will progress to clinical type 1 diabetes. However, there are reports that autoantibodies can disappear. Using data from TrialNet's Pathway to Prevention study, we aimed to determine how many multiple-autoantibody positive individuals lost autoantibodies (reversion), and test the association between autoantibody reversion and progression to clinical disease.

Research Design and Methods

Screening for islet autoantibodies was performed in 201,617 relatives of individuals with type 1 diabetes. Multiple-autoantibody positivity was defined as two or more autoantibodies on two consecutive occasions within 12 months. Subsequent reduction to one or no autoantibodies on two consecutive occasions within 12 months was considered reversion. We analyzed the effect of autoantibody reversion on progression to clinical disease using a time-dependent Cox regression analysis. Baseline characteristics of reverting individuals were compared with those who maintained.

Results

Of 3,284 multiple-autoantibody positive subjects (mean follow-up 2.2 years), reversion occurred in 134 (4.1%). Reversion was associated with reduced incidence of clinical disease; the estimated cumulative 5-year risk was 42% (95%CI 39%-45%) for the maintainers and 11% for the reverters (95%CI 6%-18%). Reversion occurred more frequently in individuals who were older (mean 19.4 vs 13.1 years old; $p< 0.001$), had lower autoantibody titers, and a lower number of positive autoantibodies (2.1 vs 3.1; $p< 0.001$).

Conclusions

Once established, it is rare for multiple-autoantibody positivity to revert. However, if reversion occurs, the risk of progressing to clinical disease is reduced. This suggests the possibility of unknown mechanisms promoting immune remission in some individuals.

F245. Single-Cell RNA-Seq Analysis of the Human Thymic Stroma Uncovers Novel Cellular Heterogeneity in the Thymic Medulla

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The thymus is a critical organ of the immune system that supports the development of diverse, self-tolerant T cells. It is comprised of multiple cell types, including developing T cells, known as thymocytes, and non-lymphoid stromal cells that promote the different stages of thymocyte differentiation. While it has been well established that thymic epithelial cells (TECs) and other stromal cells are critical for the establishment of a thymic microenvironment that supports thymopoiesis, our understanding of the mechanisms controlling the generation and maintenance of this complex tissue remains limited. To better define thymic cellular heterogeneity and gain insights into human TEC development, we thus mapped the transcriptome of individual stromal cells using single-cell RNA sequencing (scRNA-seq). We isolated and sequenced stromal cells from embryonic, postnatal, and adult tissue and used unsupervised clustering to identify populations corresponding to TECs, mesenchyme, endothelium, pericytes, mesothelium, and blood/immune cells. Analysis of soluble factors expressed by stromal cells led to the identification of candidate regulators of TEC cell fate commitment and differentiation. Sub-clustering of epithelial cells also uncovered previously uncharacterized markers of TEC subsets and revealed the presence of novel subsets of cells present in the human thymic medulla. Finally, we analyzed the expression of tissue-specific antigens to better understand how central immune tolerance is established in the human thymus. This work thus provides an invaluable platform to study thymic development and expression of antigens relevant to human autoimmune diseases.

F252. Exhaustion of the Regulatory T Cells TCR Repertoire Instigates Diabetes in NOD Mice

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Non-obese diabetic (NOD) mice spontaneously develop autoimmune diabetes. We aimed to analyze their TCR repertoire to better understand NOD autoimmunity. We performed next-generation sequencing of TCRs from splenocytes of prediabetic NOD and normal B6 mice. We separately analyzed the repertoire of CD4+ effector T cells (Teffs), $CD44^{low}$ $CD62L^{high}$ naïve regulatory T cells (nTregs) and $CD44^{high}$ $CD62L^{low}$ activated/memory Tregs (amTregs). These latter are known to respond to self-antigens and to be involved in protection against autoimmune diseases.

NOD and B6 nTreg TCR β repertoires were very diverse and mostly composed of unexpanded clonotypes. In contrast, B6 amTregs contained frequent expanded clonotypes that were lost in NOD resulting in an increased diversity of their amTregs repertoire. This was also seen, albeit to a lesser extent, in NOD Teffs. These observations suggested that NOD mice had lost the amTreg clonotypes that could protect them from diabetes.

As IL-2 administration leads to Tregs expansion and activation, and correlatively to protection from diabetes occurrence, we investigated the effects of IL-2 on NOD TCR repertoires. IL-2 allowed restauration of the amTreg expanded clones that formed dense similarity clusters. These clusters were statistically more enriched in TCRs with specificities labelled as T1D, but not as “Cancer, EAE, or SLE” obtained from a curated public database. Moreover, we could detect in these clusters islet-antigen specific TCRs defined by tetramer staining, indicating that Tregs expressing such TCRs were exhausted but not lost.

Altogether, exhaustion of islet-antigen specific amTreg instigates diabetes, and can be efficiently reverted by an IL-2 treatment.

F269. Epigenetic Regulatory T cell Quantification reveals their Diversity in Patients with Mild vs Severe IPEX Syndrome

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Primary immune regulatory disorders (PIRD) comprise inborn errors of immunity that primarily manifest as quantitative and/or functional defects of regulatory T cells (Tregs). Different mutations in the same gene can have highly variable clinical presentation, rendering the diagnosis, prognosis and treatment of PIRD challenging. Deleterious mutations in *FOXP3*, a master regulator of functional Tregs, cause IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome. While many IPEX patients have impaired Treg function, FOXP3 protein expression can range from null to above the normal range, not necessarily correlating with disease severity. Classical IPEX patients exhibit severe autoimmunity early in infancy, but recently *FOXP3* mutations have been identified in older children with atypical/milder disease. Diverse clinical phenotypes and variable FOXP3 expression make IPEX diagnosis difficult, confirming the need for novel tools to measure Tregs in IPEX and other Tregopathies.

Tregs have a unique demethylation pattern in the CNS2 promoter/enhancer region of *FOXP3* (also called TSDR; Treg–specific demethylated region) which is crucial for their stable FOXP3 expression. We previously observed that severe IPEX patients have higher TSDR demethylation compared to healthy controls. Herein, we used TSDR demethylation assay to quantify Tregs and correlate it with FACS phenotype and *in vitro* Treg function, in patients with atypical/mild IPEX vs new severe IPEX.

Epigenetically, mild IPEX patients had less elevated Tregs compared to severe IPEX, in the upper range of normal. Overall, epigenetic Treg phenotyping can precisely quantify circulating Tregs independently of protein expression, and thus facilitate the more accurate diagnosis in IPEX and other Tregopathies.

F273. MHC-mediated antigen presentation by thymic stromal cells is required to enforce central T-cell tolerance

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Abnormal selection of self-reactive T cells in the thymus is a key step to initiation of autoimmune disease.Developing thymocytes are exposed to a wide array of tissue-specific antigens (TSAs) expressed by thymic medullary epithelial cells (mTECs) under the control of *Autoimmune Regulator (Aire)*. TSA-derived peptide-MHCII complexes are displayed on mTECs or thymic DCs, and $CD4^+$ T cells that recognize these complexes are purged from the repertoire or become regulatory T cells (tTregs). Although Aire+ mTECs express high levels of MHCII, its contribution to T-cell selection is unclear. To address this, our lab has created a mouse model (*iAire-cre.MHCII$^{fl/fl}$*) to selectively and inducibly ablate MHCII in Aire-expressing mTECs that we show results in complete ablation of mTEC MHCII without affecting other thymic APC subsets or perturbing thymic homeostasis. We then used the Rip-mOVA.OTII mouse model of type 1 diabetes and found that transient loss of MHCII on mTECs led to a complete failure of negative selection of ovalbumin-specific OTII $CD4^+$ T cells, permitting their escape and activation in the pancreatic draining lymph nodes. Surprisingly, there was also a defect in intrathymic conversion of OTII $CD4^+$ T cells to $FoxP3^+$ tTregs. Similarly, in the diverse repertoire, loss of MHCII on mTECs led to a reduction in the thymic Treg pool, with FoxP3+ CD25- Treg precursors being the most severely affected. In sum, our data support a non-redundant role for direct antigen presentation by mTECs in shaping thymic T cell selection.

F276. Immunoglobulin Heavy chain profiling of early rheumatoid arthritis patients reveals the presence of highly mutated IgM and IgD expressing B cells in peripheral blood

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Background

Methotrexate is used as a first line disease modifying anti-rheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA). Although a subset of individuals may achieve durable remission, confirmation of response often requires extended monitoring, highlighting the need for predictive biomarkers to guide drug selection. Here we evaluated immunoglobulin Heavy chain (IGH) repertoire sequencing as a potential tool for predicting methotrexate response in a retrospective analysis of individuals receiving methotrexate for RA.

Methods

Patients were recruited to the Scottish Early Rheumatoid Arthritis (SERA) inception cohort. Peripheral blood RNA from 18 subjects at baseline, month 6, and month 12 post was used for IGH repertoire sequencing (Oncomine IGH-LR assay) to identify B cell clonotypes, quantify somatic hypermutation (SHM) and evaluate isotype representation. Subjects were classified as responder or non-responder per CDAI score at the 6 and 12 month timepoints.

Results

IGH sequencing analysis revealed a subset of subjects possessing an excess of IgM and IgD expressing B cells with high (>8%) SHM. Treatment with methotrexate preferentially reduced the frequency of B cells expressing switched isotypes (IgG, IgA, IgE) compared to those expressing IgM or IgD ($p = 0.003$, Wilcoxon).

Conclusion

These results provide insight into the mechanism of action of methotrexate and suggest a potential role for highly mutated IgM and IgD expressing B cells in RA. Ongoing studies will clarify the potential biomarker utility of IGH sequencing in RA.

F277. Systemic lupus erythematosus: can a monogenic cause explain the phenotype?

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BACKGROUND:
Monogenic lupus provides significant insight into the cause and mechanisms of lupus.
We describe a patient with monogenic lupus.

CASE REPORT:
We present the case of 15-year-old boy without family history of consanguinity. His mother had an abortion and his sister died one hour after birth.
He had a history of neonatal sepsis, failure to thrive, muscular hypotonia, abnormal facies, microcephaly and arthrogryposis.
At 4 years of age he presented discoid skin rash, arthritis, oral ulcers, alopecia and acute glomerulonephritis that required hemodialysis for several months. At that time, the disease was complicated with 2 episodes of pneumonia, while receiving immunosuppressant drugs.
Six years later, his renal function started to go down again, He presented severe ascites. Despite treatment with Rituximab, Mycophenolate Mofetil and steroids, the kidneys were severely damaged, so the patient started hemodialysis again.
At 13 years old I received a successful kidney transplantation. However, 3 years later lupus became active again, with kidney and nervous system affectation (peripheral neuropathy).

Basic immunologic testing (WBC, IgG, IgA, IgM, T-cell, B-cell and NK-cell counts) before Rituximab were within normal ranges, except for reduced levels of complement proteins C3 and C4.
The interferon signature was positive, identifying increased expression of 11 genes induced by Interferon
Whole exome sequencing performed in Texas (USA) revealed two pathogenic mutation in DNASE1L3 gene.

CONCLUSIONS:
Our finding confirm the critical role of impaired clearance of degraded DNA in SLE pathogenesis.
This is the first reported cases of DNASE1L3 gene mutation in Peru.

F278. Determining the Role of IgA and FcαR in Plasmactyoid DC (pDC) Immune Complex Activation in Systemic Lupus Erythematosus (SLE)

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Systemic lupus erythematosus (SLE) is a heterogenous autoimmune disease characterized by the presence of circulating autoreactive anti-nuclear antibodies (ANAs). ANAs form immune complexes (ICs) upon binding to nuclear antigen and these ANA-ICs promote a feedforward loop by enhancing immune responses in cells that recognize ANA-ICs via Fc receptors (FcRs). Plasmacytoid dendritic cells (pDCs) are FcR expressing cells that promote SLE pathology. ANA-ICs deliver nucleic acids to endosomal TLR7 and TLR9. These TLRs then induce type I IFN secretion and immune activation upon nucleic acid recognition. SLE patients have ANAs with different reactivities to nuclear antigens that can correlate to disease manifestations. However, antibodies differ by reactivity and isotype. IgG isotype facilitated ANA-IC internalization via FcgRIIA has been the most studied antibody-mediated route for IFNα production in pDCs. IgA isotype ANAs remain under investigated despite being present in half of SLE patients. We show here the novel result that human pDCs express the IgA-specific FcαR (CD89) by flow cytometry. Additionally, in a preliminary study we showed that pDCs from SLE patients express increased FcαR compared to pDCs from healthy controls. Strikingly, we also found that IgA autoantibodies were a critical component of pDC ANA-IC activation when these ANA-IC were generated with serum from IgA autoantibody positive SLE donors complexed with smRNP nuclear antigen. Therefore, our study shows that IgA-containing immune complexes and FαRI critically contribute to pDC type I IFN production in SLE.

F279. Ankyrin G autoantibodies are associated with steroid-responsive encephalitis

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However, clinical decision-making in the setting of suspected, yet seronegative autoimmune encephalitis remains a challenge. Because early diagnosis and treatment improves outcomes, AE-associated autoantibody biomarker discovery can reduce morbidity and mortality. Common CNS autoantibody discovery approaches, for example live neuron immunostaining and immunoprecipitation mass spectrometry, are low-throughput and test for a fraction of possible antigens. We therefore used a whole human proteome phage display immunoprecipitation sequencing platform (PhIP-Seq) as an unbiased screen for novel autoantibodies in steroid-responsive autoimmune encephalitis. By integrating anatomic tissue staining with PhIP-Seq, in two individuals with AE we identified autoantibodies targeting distinct eptiopes mapping to the N-terminal membrane-binding domain of the submembranous protein Ankyrin G (AnkG). AnkG is enriched in the nervous system and anchors voltage gated sodium channels to the axon initial segment and nodes of Ranvier for the initiation and propagation of action potentials respectively. In one case, the autoantibody response was exclusive to the PhIP-Seq identified epitope. In the second case, we used an isoform-specific AnkG deletion mutant to show that the putative epitope is likely also restricted to the PhIP-Seq identified epitope. While other nodal and paranodal autoantibodies have been described in central and peripheral autoimmune neurologic disorders, to our knowledge this is the first description of anti-AnkG antibodies in autoimmune neurologic disease. Given that AnkG is an intracellular protein, it is unclear whether these autoantibodies are directly pathogenic. This work highlights the value of PhIP-Seq for both antigen discovery and epitope mapping.

F280. Processing-Resistant NF-kB2/p100 variants Disrupt T-Cell Tolerance and Cause Autoimmunity

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NF-kB2/p100 (p100) has dual functions as an inhibitor of NF-kB (IkB) and as a precursor of the active NF-kB2/p52 (p52) transcription factor. *NFKB2* mutations cause immunodeficiency and autoimmunity in humans, but mechanisms underlying the immune dysregulation remain unclear. By studying an allelic series of mice with distinct *Nfkb2* mutations and humans with rare *NFKB2* mutations we found that excessive accumulation of p100 was sufficient to diminish thymocyte deletion in a T-cell-extrinsic mechanism and impair Foxp3$^+$ T-regulatory cell differentiation in a T-cell-intrinsic mechanism. Multi-organ autoimmunity, which invariably affected the exocrine pancreas, developed in mouse strains with increased expression of p100 combined with decreased p52, but not in strains with deficiency of both p100 and p52. Repertoire analysis by sequencing revealed that self-reactive T cell receptor motifs were

increased in strains that developed autoimmunity and in patients with pathological *NFKB2* mutations. p100 variants that resist cleavage and degradation reveal that T-cell tolerance and susceptibility to autoimmunity are exquisitely sensitive to p100 abundance.

F293. Extremely early-onset type 1 diabetes is associated with low levels of islet-specific IFN-γ and IL-17A responses but increased frequencies of circulating activated follicular helper T cells

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Type 1 diabetes is very rare in infancy, and not frequently diagnosed under the age of two years. Individuals with extremely early-onset type 1 diabetes (EE-T1D) might have very aggressive autoimmunity, which could explain why they develop the disease earlier in life. The current study aims to investigate the role of the immune system in the pathogenesis and progression of beta cell destruction through the study of EE-T1D individuals. Subjects with specific monogenic autoimmunity and polygenic disease were the main cohorts of the study. Methodology included deep immunophenotyping using multiparameter flow cytometry covering populations from the innate and the adaptive immune system and assessment of antigen-specific responses via triple colour fluorospot (IFN-γ/IL-10/IL-17A). Hypothesis driven analysis of the flow cytometry data identified higher frequencies of circulating activated follicular helper T cells (CD4+CXCR5+PD-1+ICOS+ conventional memory T cells) in EE-T1D individuals when compared to newly diagnosed T1D patients. Islet-specific responses showed low reactivity in the peripheral blood of EE-T1D. Responses against proinsulin were found in 31% of EE-T1D individuals for IL-17A and 19% for IFN-γ as compared to adult onset T1D subjects where responses were found in more than 50% of individuals. These data have identified a cellular immune signature specific to EE-T1D patients that may contribute to the early disease onset. Taken together, this study will enable potential targets for treatment to be identified, as well as pathways pivotal to beta cell destruction, with a view to determine biomarkers of disease progression and potential key drivers of T1D pathogenesis.

F296. T cell response phenotypes in Type I Diabetes characterized by single cell analysis

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Type 1 Diabetes (T1D) is characterized by the presence of autoreactive T cells at the site of β cell destruction. $CD4^+$ T cells play a key role in coordinating the auto-antigen specific response of the immune system. These cells can be detected in blood circulation and changes in their number and their phenotype have become a paradigm for successful therapy to prevent immune mediated destruction of insulin

producing β cells. Modulating the transcriptional profiles of auto-antigen specific T cells before T1D clinical onset using immune modulators may prevent autoimmunity, by altering the response and/or transcriptional profiles of auto-antigen specific $CD4^+$ T cells or by affecting priming ability of $CD4^+$ naïve cells. We used staphylococcal enterotoxin B (SEB) and in vitro proliferation assays as a model to induce and modulate TCRVβ-specific naïve or memory $CD4^+$ T cell responses and we have identified three compounds that successfully reduced TCRVβ-specific response to SEB. Transcriptomic analysis on the single cell level with RNA sequencing will characterize these $CD4^+$ T cell signatures. Application of this knowledge in auto-immunity may then contribute to either induction of tolerance to autoantigens in a therapeutic setting or to imprint tolerant profiles to islet-directed $CD4^+$ T cells, and establish potent preventive therapies for T1D and other autoimmune diseases.

F298. IL-6 mediated pSTAT1 responses and their role in CD4+ T cell fate decision and function

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IL-6 is a pleiotropic cytokine involved in various cellular, metabolic, and pro-inflammatory processes that collectively impact the phenotype and function of diverse immune cells. IL-6 signaling has been studied extensively in the context of CD4+ T cells, where it drives polarization towards certain Th cell lineages (Th17, TFH) and resistance of effector T cells against suppression by Treg cells. The effect of enhanced or deregulated IL-6 signaling has been implicated in several autoimmune diseases including T1D, RA or MS. To elucidate the role of enhanced IL-6 signaling in determining the fate of CD4+ T cells, we studied a genetic variant of the IL-6R gene locus, rs2228145 (Asp358Ala), which causes strikingly different levels of membrane-bound IL-6R between carriers of the different alleles. We showed that increased surface expression of IL-6R in healthy subjects carrying the major variant (AA, Asp358Asp) correlated with enhanced signaling via pSTAT1 in response to IL-6. This effect was enriched in central memory cells, which displayed the highest difference in mbIL-6R expression between genotypes. Using transcriptomics, publically available data and flow cytometry we confirmed enrichment of a pSTAT1-driven transcriptional gene network in the major genotype and showed IL-6 dependent induction of target genes including BCL-6 and ICAM-1. Our work highlights how enhanced IL-6 signaling shifts the balance between pSTAT3/pSTAT1 towards pSTAT1, giving us a platform to study the mechanisms underlying the potential IL-6 dependent rise of pathogenic T cell responses involved in autoimmunity.

F308. Exploring the potential of Siglec-10 targeting in systemic lupus erythematosus (SLE)

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Systemic lupus erythematosus (SLE) is a complex multisystem autoimmune disease with high morbidity. The pathogenesis of SLE is incompletely understood, however, it is clear that cytokine imbalances contribute to immune dysfunction, trigger inflammation and induce organ damage. In this regard, several cytokine-targeted inhibitors have reached clinical trials. However, their success may be limited due to a multiplicity of pathogenic mechanisms that cause disease. Therefore, approaches to more broadly target the Pattern Recognition Receptors (PRR) family members implicated in SLE may have greater therapeutic benefit.

In this study, we examined RNA expression of Sialic acid-binding immunoglobulin-type lectins-10 (Siglec-10) on peripheral blood cells from a heterogeneous cohort of SLE donors (n=28) and age and sex-matched healthy controls (n=17). Analysis revealed that Siglec-10 expression was significantly lower in SLE patient PBMCs compared to healthy controls. To test if reduced Siglec-10 levels might impact on PRR-induced cytokine production, and thereby contribute to SLE-associated inflammation, we generated Siglec-10 deficient human monocytic cells using CRISPR-Cas9 gene targeting. Interestingly, Siglec-10 deletion resulted in significantly elevated production of TNF, IL-6 and IL-1b in response to PRR ligands when compared with WT control cells, at both the mRNA and protein levels. Further, our detailed molecular analysis suggests that the activation of Siglec-10 specifically inhibits PRR-induced NF-kB and MAPK pathways to repress cytokine production.

Collectively, our evidence suggests the Siglec-10 are likely to play key role in modulating immune cell activity and the production of inflammatory cytokines, and that its differential expression in SLE may significantly impact SLE disease activity.

F324. Distinctive binding properties of patient-derived monoclonal LGI1-autoantibodies determine pathogenicity

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Autoantibodies against leucine-rich glioma inactivated 1 (LGI1), a secreted neuronal protein, can be found in patients with autoimmune encephalitis, frequently presenting with seizures and amnesia. LGI1 is composed of two major domains, the leucine rich repeat (LRR) and epitempin (EPTP) domain and is known to bind its interaction partners via the EPTP domain. Here, we aimed to study epitopes and pathogenicity using patient-derived LGI1-specific monoclonal antibodies (mAbs).

LGI1-specific mAbs were generated from peripheral B cells of patients with LGI1 autoantibodies. mAbs were characterized by their sequences, binding strengths and their epitopes, in both HEK293T cells and cultured rat hippocampal neurons. Bilateral stereotactic injections into the hippocampus of mice were performed to assess their pathogenicity.

Fourteen mAbs from two patients were generated. Sequence analysis revealed structural heterogeneity and high mutation frequencies. In contrast to polyclonal serum reactivities in patients, the 14 mAbs recognized either the LRR or EPTP domain. Interestingly, only LRR-specific mAbs recognized LGI1 bound to its interaction partners, and induced internalization of their target *in vitro*. In contrast, EPTP-specific mAbs predominantly blocked the interaction of LGI1 with its receptors. Both specificities were capable of inducing memory impairment after stereotactic injections into the hippocampus of mice.

Patient-derived LGI1-specific mAbs have revealed distinct LGI1-specificities, with different molecular mechanisms partly attributable to their epitopes and affinities. Both specificities coexist in individual patients and show pathogenic potential with memory impairment *in vivo*.

F335. Single-cell repertoire tracing identifies rituximab refractory B cells during myasthenia gravis relapses.

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Rituximab, a B cell-depleting therapy, is indicated for treating a growing number of autoantibody-mediated autoimmune disorders. However, relapses can occur after treatment and autoantibody-producing B cell subsets may be found during relapses. It is not understood if these autoantibody-producing B cell subsets emerge from the failed depletion of pre-existing B cells or are generated *de novo*. To further define the mechanisms that cause post-rituximab relapse, we studied patients with autoantibody-mediated muscle-specific kinase (MuSK) myasthenia gravis (MG) who relapsed after treatment. We carried out single-cell transcriptional and B cell receptor (BCR) profiling on longitudinal B cell samples. We identified clones present prior to therapy that continued to persist during relapse. Persistent B cell clones included both antibody-secreting cells and memory B cells characterized by gene expression signatures associated with B cell survival. A subset of persistent antibody-secreting cells and memory B cells were specific for the MuSK autoantigen. These results demonstrate that rituximab is not fully effective at eliminating autoantibody-producing B cells and provide a mechanistic understanding of post-rituximab relapse in MuSK MG.

F418. Autoreactivity of EBI2+ B cell is Positively Correlated with Type 2 Inflammation in Chronic Rhinosinusitis with Nasal Polyps

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Background: We have previously found that anti-dsDNA IgG autoantibodies are increased in the tissue of patients with Chronic Rhinosinusitis with Nasal Polyps (CRSwNP). Expression of the Epstein-Barr virus-induced protein 2(EBI2) is critical for B cell localization to extrafollicular sites. We have reported that EBI2+ B cells were elevated in NP, and they were antibody secreting cells (ASCs). In this study, we investigated whether anti-dsDNA IgG ASCs are EBI2+ and their association with cytokines and inflammatory mediators in CRSwNP.

Methods: Flow cytometry and sorting and ELISpot were utilized to compare the frequency of total IgG and dsDNA IgG ASCs among EBI2+ and EBI2- B cells. Luminex assay and scRNA-seq analysis were utilized to evaluate the relationships between dsDNA-IgG, EBI2 expression and key inflammatory mediators.

Results: Although tonsils had 6.3-fold more frequent B cells than NP, those from NP were more frequently CD27+CD38+ plasmablasts than tonsils. In analysis of ASCs sorted from NP (n=4) and tonsil (n=7), we found anti-dsDNA IgG ASCs were more frequent among EBI2+ cells compared to EBI2- cells with 34- and 8.6- fold increases in dsDNA reactivity ($p< 0.05$), respectively. Anti-dsDNA-IgG in polyps was positively correlated with IL-4, IL-5, and IL-13 ($r >0.6$, $p< 0.05$) and average cellular EBI2 expression by scRNA-seq ($r=0.6$, $p=0.02$).

Conclusions: Although B cells are significantly more abundant in tonsils, NP derived B cells more frequently expressed dsDNA-specific IgG. These differences appear to occur primarily in EBI2+ B cells. In NP tissue, EBI2 expression and anti-dsDNA IgG expression were associated with type 2 cytokines.

F423. Multiparameter flow cytometry analysis of blister biopsies from morphea and dermatomyositis reveals immune abnormalities in nonlesional skin that may precede clinical involvement

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Dermatomyositis (DM) and morphea (localized scleroderma) are rare connective tissue diseases that are characterized by a variable degree of skin manifestations and fibrosis, respectively. We applied high-parameter flow cytometry (Cytek Aurora) to analyze the inflammatory differences in suction blistering samples of lesional and nonlesional skin in a patient with DM and a patient with morphea. In DM, the lesional skin infiltrates contained blasted lymphocytes and natural killer (NK) cells. More CD38 was expressed on lesional CD4+ and CD8+ T cells compared to nonlesional skin by both median fluorescence intensity (MFI) and percent. The lesional B cells expressed CD38, CD69, CD103, or Fas, while nonlesional B-cells expressed a low level of Fas. CD56+ CD16+ NK cells were only found in lesional skin. Lesional monocytes, B cells, and plasmacytoid dendritic cells (pDCs) expressed more HLA-DR than nonlesional cells. In the morphea biopsies, CD3+ T cells were present in nonlesional and lesional skin. Lesional CD4+CD8+ T cells expressed CXCR3, CD69, or CD103. There were CD38+ and CD103+ NK cells in lesional skin and blood, but none in nonlesional skin. The monocytes in lesional skin and blood had similar HLA-DR expression, while nonlesional monocytes had virtually no HLA-DR.

Lesional skin pDCs had more HLA-DR vs. nonlesional skin. In conclusion, our case studies revealed abnormalities even in nonlesional skin of morphea and DM that may play a role in inflammatory sequella in patients with rare connective tissue disorders.

F424. Conventional Dendritic Cell Subsets in Cutaneous Lupus Erythematosus and Dermatomyositis

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Given their ability to bridge innate and adaptive immunity, dendritic cells (DCs) play an important role in the promotion of autoimmunity. Recently, the diversity and complex roles of various DC subsets to disease heterogeneity in cancer and autoimmunity have been recognized. We sought to characterize the frequencies of various DC subsets, particularly conventional DCs (cDCs) such as cDC1s and cDC2s, in cutaneous lupus erythematosus (CLE) and dermatomyositis (DM). We performed immunofluorescence on treatment-naïve biopsies from patients with CLE or DM. Tissues were stained with fluorescently tagged anti-CLEC9A and anti-CLEC10A antibodies to identify cDC1 and cDC2s respectively. The number of cDC subsets were counted across three high-power fields. To characterize the inflammatory profile, we performed fluorescence *in situ* hybridization (FISH) and Imaging Mass Cytometry using cDC subset specific and IL17, IL4, and IFNy probes. Patients with CLE were stratified according to their response to hydroxychloroquine (HCQ), dual HCQ and quinacrine (QC), or antimalarial nonresponders (NR). Our data show a significant increase in cDC2s in CLE ($p< 0.05$) and DM ($p< 0.01$) compared to healthy controls (HC). Patients with CLE who responded to QC demonstrated a significant increase in cDC2s as well, compared to HC ($p< 0.01$). FISH and IMC revealed cDC2 production of IL17. Our findings suggest a potential role for cDC2s in CLE and DM. cDC2s may also contribute to HCQ refractoriness and may be more responsive to QC. More research is needed to clarify the role of cDC subsets in autoimmunity and their modulation of patient responses to therapeutics.

F429. Sodium chloride generates anti-inflammatory Th17 cell responses but amplifies Th17 cell pathogenicity in pro-inflammatory cytokine microenvironments

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T helper cells integrate signals from their microenvironment to acquire distinct specialization programs for efficient clearance of diverse pathogens or for immunotolerance. Ionic signals have recently been demonstrated to affect T cell polarization and function. Sodium chloride (NaCl) was proposed to accumulate in peripheral tissues upon dietary intake and to promote autoimmunity via the Th17 cell axis. Here we demonstrate that high NaCl conditions induced a stable, pathogen-specific, anti-inflammatory Th17 cell fate in human T cells *in vitro*. The p38/MAPK pathway, involving NFAT5 and SGK1, regulated FoxP3 and interleukin (IL)-17-expression in high-NaCl conditions. The NaCl-induced

acquisition of an anti-inflammatory Th17 cell fate was confirmed *in vivo* in an experimental autoimmune encephalomyelitis (EAE) mouse model, which demonstrated strongly reduced disease symptoms upon transfer of T cells polarized in high NaCl conditions. However, NaCl was coopted to promote murine and human Th17 cell pathogenicity, if T cell stimulation occurred in a pro-inflammatory and TGF-b-low cytokine microenvironment. Taken together, our findings reveal a context-dependent, dichotomous role for NaCl in shaping Th17 cell pathogenicity. NaCl might therefore prove beneficial for the treatment of chronic inflammatory diseases in combination with cytokine-blocking drugs.

F435. Cannabidiol Facilitates the Development of Autoimmune Th17 Cells

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Introduction: The legalization of cannabinoids for medical use has reinforced their emerging role as a treatment of chronic pain in patients with rheumatic diseases. While an immunosuppressive effect of cannabinoids has been postulated, it could not be confirmed in clinical trials, so far. The aim of this study was to evaluate possible immune-modulatory effects of cannabinoids in patients with rheumatic autoimmune diseases.

Methods: We analyzed the influence of CBD on Th17 cell differentiation and studied ex vivo Th17 cell frequencies in patients consuming cannabidiol (CBD) oil. Samples from 46 patients with rheumatoid arthritis, psoriatic arthritis or systemic lupus erythematosus and from 13 healthy individuals were analyzed.

Results: Differentiation of Th17 cells from autoimmune CD4+ T cells is significantly enhanced in vitro by CBD (6.5±0.5% vs. 3.3±0.2%; $p< 0.0001$). In striking contrast, Th17 cell differentiation is reduced by CBD in healthy individuals (1.9±0.6% vs. 3.6±0.3%; $p=0.0002$). Moreover, treatment with CBD increases Th17 cell frequencies in the peripheral blood of patients with rheumatic autoimmune diseases (1.1±0.3% vs. 4.5±1.3%). Interestingly, CBD induces SGK1, an important regulator of pro-inflammatory Th17 cells. The observed effects are independent of CB1/CB2/GPR55 expression, suggesting that CBD activates autoimmune Th17 cells through other receptors that are not expressed on Th17 cells from healthy individuals. Finally, CBD has a significant impact on the viability of CD4+ T cells.

Conclusion: CBD promotes the differentiation of autoimmune Th17 cells while it inhibits Th17 cells from healthy individuals. Our data suggest that CBD should be used with caution in patients with autoimmune diseases.

F448. Systemic Depletion of CD4 T Cells Ameliorates Neuropsychiatric Lupus

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Autoreactive T cells, particularly CD4+ T cells, are critical in the pathogenesis of systemic lupus erythematosus (SLE), through cytokine amplification, providing B cell help, and tissue damage via aberrant homing. However, despite the evidence that T cells, particularly effector CD4+ T cells, prominently infiltrate the choroid plexus and other brain regions in murine SLE models and in some human lupus patients with neuropsychiatric disease, T cells' contribution to mechanisms of neuropsychiatric manifestations has not been carefully evaluated. We investigated whether systemically depleting CD4+ T cells ameliorates neurobehavioral deficits in murine lupus. For 10 weeks, weekly 1 mg intraperitoneal injections after an initial 2 mg bolus of anti-CD4 (clone GK1.5, n=15), IgG2b isotype control (n=14), or PBS (n=7) were administered to 6-week old female MRL/lpr mice. Mice subsequently underwent behavioral assessments and systemic disease was evaluated. Administration of the anti-CD4 antibody to MRL/lpr mice successfully depleted CD4+ T cells, and significantly ameliorated systemic disease as indicated by decreased splenomegaly and lower anti-dsDNA titers compared to the PBS and isotype control antibody groups. Furthermore, spatial memory was significantly improved in CD4-depleted mice, as well as moderated improvement in depressive-like behavior. Additionally, histological evaluations demonstrated amelioration of the choroid plexus infiltrate, particularly T cells, in anti-CD4 treated mice. Our results indicate that T cells do, in fact, contribute to the pathogenesis of neuropsychiatric manifestations in murine NPSLE and further studies are needed to evaluate their direct contributions to NPSLE.

F450. Extrafollicular Compartment Alterations in Juvenile Dermatomyositis Blood

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OBJECTIVE: Juvenile Dermatomyositis (JDM) is the most common pediatric inflammatory myopathy. We used flow cytometry to examine the blood B and T-cell compartments in search of pathogenic clues and markers of disease activity.

METHODS: Two cohorts of JDM patients were recruited, clinical measures of disease activity, including the Manual Muscle Testing (MMT-8), were recorded and blood was collected for flow cytometry studies. Samples from 35 JDM patients and 10 healthy controls were first examined using an 11-color flow cytometry panel. A validation cohort included 25 JDM patients and 5 healthy controls. Samples were phenotyped using an extended 25 color flow panel and disease activity data was included in the analysis.

RESULTS: A significant decrease in both follicular and extra-follicular Th1/17 cells and an expansion of $CXCR5^-$ Th2 cells was observed in both cohorts. Extrafollicular ($CXCR5^-$) switched memory (EFSM) B cells ($CD20^+$ $CD27^+$ IgD^-) were expanded in the training cohort ($p< 0.01$) and showed a positive trend in the second cohort, especially when comparing patients with active disease with those in remission and healthy controls ($p=0.06$). Importantly, in both cohorts the expansion of EFSM B cells associated with

worse (lower) manual muscle testing score (MMT-8) (test cohort Spearman's rho=-0.56, p=0.01; validation cohort -0.60, p=0.04).

CONCLUSIONS: Children with JDM show alterations in their extrafollicular B and T-cell compartments. Expansion of a novel population of EFSM B-cells is associated with muscle disease activity.

TH3. Critical Role of the Regulatory Cytokine IL-34 in Treg Function

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Immune homeostasis requires competent Tregs that must maintain a stable phenotype to control autoimmunity. IL-34 is a cytokine that binds to CSF-1R (CSF-1 receptor), CD138 and PTPζ and first described as mainly involved in myeloid cell survival and differentiation. However, we recently demonstrated the expression of IL-34 by activated human FOXP3$^+$CD4$^+$ and CD8$^+$ Tregs and in a rat model of transplantation tolerance. Additionally, its overexpression using an AAV-IL-34, induces long-term allograft tolerance in the rat through the modulation of monocytes into pro-regulatory M2 macrophages and CD8$^+$ and CD4$^+$ Tregs induction. To understand the role of IL-34 in the development and immune responses, we generated *Il34*$^{-/-}$ rats using the CRISPR/Cas9 technology. We report that *Il34*$^{-/-}$ animals showed unstable proinflammatory phenotype exacerbated under inflammatory conditions. We show that *Il34*$^{-/-}$ animals develop a more severe EAE and TNBS-colitis compared to *Il34*$^{+/+}$ controls. Moreover, we demonstrated that *Il34*$^{-/-}$ CD4$^+$ Tregs failed to protect *Il2rg*$^{-/-}$ rats from wasting disease (disease induced by the injection of Teff) in contrast to *Il34*$^{+/+}$ CD4$^+$ Tregs, underlying the essential role of IL-34 in the Treg suppressive function. Finally, we showed the regulatory potential of recombinant human IL-34 in association with a sub-optimal dose of rapamycin to delay xenogeneic GVHD development and human skin allograft rejection in immune humanized immunodeficient NSG mice models. Altogether, our data unravel the crucial role of IL-34 in CD4$^+$ Treg suppressive function and its therapeutic potential in transplantation.

TH8. Multi-parametric Interrogation of the Systemic Lupus Erythematosus (SLE) Immunome Reveals Multiple Derangements

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease best interrogated with multi-parametric, holistic approaches such as mass cytometry (CyTOF). We hypothesize that significant differences exist between immunomes of newly diagnosed SLE and healthy subjects.

CyTOF data from 5 SLE subjects (median age 125 months) was uploaded to an online analytical platform, the Extended Polydimensional Immunome Characterization(EPIC) discovery tool, for comparison to its database (51 age-matched controls) via normalization and FlowSOM clustering to 50 nodes with 37 markers. The Mann-Whitney U test identified significantly different cluster frequencies.

Correspondence analysis comparing global differences in cluster frequencies showed segregation of SLE subjects away from healthy controls. Multiple significant differences were identified ($p< 0.05$). Notably, a memory CD4+CD152+PD1+ T-cell subset (CD4+CD152+PD1+CD45RO+CD25-FoxP3-) was enriched in SLE (median: 2.17%, interquartile range: 1.66 to 7.74% of CD45+ peripheral blood mononuclear cells) versus control (1.34%, 1.06-1.58%; $p = 0.00267$). Expression of known checkpoint inhibitors (PD1,CD152) could be important for SLE immunopathogenesis.

Secondly, the innate lymphoid cell 2 (ILC2) subset (Lin-CD7+CD25+CD127+GATA3+) was markedly depressed in SLE (0.11%, 0.1-0.255%) versus control (0.41%, 0.25-0.55%; $p = 0.0293$). ILC2s protect epithelial integrity and a reduction suggests impaired protective roles in SLE.

Supervised cell frequencies from bivariate analysis correlate strongly with unsupervised cell frequencies, validating these results (Pearson's correlation coefficient $r = 0.9926$, $p < 0.001$ (CD4+CD152+PD1+CD45RO+CD25-FoxP3-); $r = 0.8863$, $p < 0.05$ (ILC2)).

With a multi-parametric unbiased approach comparing SLE subjects to a large database of age-matched healthy controls, we identified two immune subsets of potential immunopathogenic importance for further studies.

TH11. Systemic Sclerosis Perturbs the Architecture of the Immunome

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Systemic sclerosis (SSc) is an autoimmune disease characterised by excessive fibrosis of skin and internal organs, and vascular dysfunction. Association of T and B cell subsets have been reported in SSc, however there is lack of systematic studies of functional relations between immune cell subsets in this disease. This lack of mechanistic knowledge hampers targeted intervention. In the current study we ought to determine differential immune cell composition and their interactions in peripheral blood of SSc patients and its impact on disease severity and progression. Mononuclear cells from blood of SSc patients (n=20) and healthy controls (n=10) were analysed by mass cytometry using a 36 marker (cell-surface and intracellular) panel. Transcriptome analysis (m-RNA sequencing) was performed on sorted T and B cell subsets. Unsupervised clustering analysis revealed significant differences in the frequencies of T and B cell subsets in patients. Correlation network analysis highlighted an overall dysregulated immune architecture coupled with domination of inflammatory senescent T cell modules in SSc patients. Transcriptome analysis of sorted immune cells revealed an activated phenotype of CD4 and MAIT cells in patients, accompanied with increased expression of inhibitory molecules, reminiscent of phenotype exhibited by functionally adapted, exhausted T cells in response to chronic stimulation. Overall this study provides an in depth analysis of systemic immunome in SSc, highlighting role of inflammation and chronic stimulation mediated exhaustion and functional adaptation of immune cells, with implications to delineate mechanism of pathogenesis and identify diagnostic/therapeutic targets.

TH14. Identifying changes in peripheral lymphocyte subpopulations at the onset of adult type 1 diabetes and their long-term evolution

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T- and B-lymphocytes play an important role in the pathogenesis of type 1 diabetes (T1D). Flow cytometry allows their characterization in peripheral blood, letting to investigate changes in cellular subpopulations that can provide insights in T1D pathophysiology.
CD4+ and CD8+ T cells (including naïve, central memory, effector memory and terminally differentiated effector (TEMRA), Th17 and Tregs) and B cells (naïve, unswitched memory, switched memory and transitional B cells) were analysed in peripheral blood of adult T1D patients at onset (n=35) and after ≥2 years (n=11) using multiparametric flow cytometry. Sex and age matched HD (n=40) were used as controls.
Here we report a decrease in the percentage of early and late effector memory CD4+ and CD8+ T cells (TCD4+:p< 0.001 and p< 0.001, TCD8+:p=0.027 and p< 0.001). In contrast, percentage and absolute numbers of naïve CD4+ and CD8+ T cells (TCD4+:p=0.008 and p=0.0241, TCD8+:p=0.002 and p< 0.001) were increased. Moreover, an increase in the percentage of total and transitional B cells was

found at onset (p=0.009 and p< 0.001). Regarding Tregs, we observed a decrease in the percentage of memory and activated Treg subsets (p< 0.001).
After 2 years follow-up this profile was maintained with the exception of an increase in the percentage of IgM memory B cells (p=0.003) compared with baseline was found.
In conclusion, the observed changes in the percentage and/or absolute number of lymphocyte subpopulations of adult T1D patients support the hypothesis that effector cells migrate to the pancreas and this autoimmune process perseveres along the disease.

TH26. Performance Summary for Multiplexed, Isotype-Specific Research-Use Serology Assays for Detection of Autoimmune Reactivities

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Background: Autoimmune diseases affect over 50 million Americans. The presence of specific autoantibodies can predict disease onset in at-risk individuals (e.g., type 1 diabetes, systemic lupus erythematosus, and celiac disease), and assist in distinguishing disorders with similar clinical features (e.g., type 1 versus type 2 diabetes). Multiplexed serology panels were developed for profiling IgG, IgA, and IgM autoantibody responses against 24 different autoantigens associated with important autoimmune diseases or connective tissue disorders (72 assays in total). These research-use-only panels were developed on the sensitive MSD® MULTI-ARRAY technology platform and included measurements using bridging and/or classical serology assay formats. Serum-derived calibrators were used for quantitative measurement of each reactivity and as positive/negative controls for assay performance tracking. These panels were applied to three sample sets: (1) samples from a drug trial (T1DAL) for type 1 diabetes (ITN), (2) samples from a clinical study on gluten-free diets for celiac disease (Harvard University), and (3) matched lupus disease samples from individuals at or without flare (University of Minnesota).

Results: Assay performance data for calibrators, controls, and test samples are presented to demonstrate the reproducibility and robustness of the assay methods. Selected data are shown for markers that distinguish subgroups within a study set.

Conclusion: The multiplexed isotype-specific autoantibody assays provided reliable, quantitative, and sensitive measurement of 72 specific reactivities while requiring less than 200 µL of serum/plasma per sample. This platform provides a new tool that can be used in autoimmune disease research to broadly profile autoimmune reactivities in each sample.

TH31. Serum sCTLA-4 level is not associated with type 1 diabetes or coexistence of autoantibodies in children and adolescent patients from the southern region of Saudi Arabia

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Introduction

The soluble form of CTLA-4 is associated with several autoimmune diseases. The aim of the study is to look for the level of sCTLA-4 among T1DM patients and to identify a possible association with autoantibody coexistence.

Methods

Two hundred two T1DM patients (1-17 years old, median age 11 years) were enrolled in the study. Of these T1DM patients, 142 were selected for further investigation. Fifty of the selected patients were serologically positive for co-existing autoantibodies, and 105 individuals were used as non-diabetic controls. Anti-human sCTLA-4 and GAD/IA2 IgG were measured for all subjects using commercially available ELISA tests. Standard statistical analysis was conducted as required.

Results

Ninety-four (66%) of the 142 T1DM patients and five subjects (4.7%) in the non-diabetic group had antibodies positive for GAD/IA2 IgG. Serum sCTLA-4 was low in both the diabetic and control groups ($p = 0.10$). Seven patients (4.9%) in the T1DM group had high levels of sCTLA-4; two of them were double-positive for anti-TPO and anti-TG antibodies. In the control group, nine individuals (8.6%) were positive for sCTLA-4. Among the T1DM patients, no significant relationships were observed between sCTLA-4 level and age of onset ($p = 0.43$), disease duration ($p = 0.09$), or glycemic control ($p = 0.32$).

Conclusion

Despite the previous findings of high of sCTLA-4 levels in autoimmune diseases, serum level of sCTLA-4 is not significantly different in T1DM patients compared to that in non-diabetic adolescents. Furthermore, we did not observe any association with autoantibody presence, glycemic control, or disease duration.

TH34. Clonal expansion of T cells in the cerebrospinal fluid of multiple sclerosis patients

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T cells are believed to be key mediators in multiple sclerosis (MS) immunopathogenesis, however, it is not clear which T cells are likely relevant to the disease. Cerebrospinal fluid (CSF) provides a window to the inflammatory response in the central nervous system (CNS). In this study, we sought to comprehensively analyze the T cell repertoire within the CSF of MS patients and to determine if certain clonotypes predominate. Single cell RNA-Seq paired with T cell receptor (TCR) sequencing was performed on T cells from the CSF of a cohort of newly diagnosed, untreated MS patients and compared to patients with other neuro-inflammatory conditions and healthy controls. In a number of MS patients, we identified T cells that were highly clonally expanded in the CSF relative to the blood. The majority of uniquely expanded T cell clonotypes in the CSF were CD8+ and exhibited an antigen-

experienced and cytotoxic profile. These findings provide important insight into the repertoire of central nervous system-infiltrating T cells likely important to MS.

TH38. Refining the HLA-Disease Association Landscape in Neuromyelitis Optica Spectrum Disorders (NMOSD)

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We conducted case-control HLA association analyses of NMOSD cases and ethnically matched healthy controls (HCs) from the U.S. (European American Cases=275 (72% anti-AQP4+), HCs=2248; African American Cases=72 (89% anti-AQP4+), HCs=150; and US Hispanics Cases=69 (86% anti-AQP4+), HC=179) and also from Japan (Cases=48 (100% anti-AQP4+), HCs=63).In accordance with previous findings, the results show that alleles carried on the DRB1*03:01:01:01 ancestral 8.1 haplotype confers significant susceptibility across all ancestral groups examined, with highly significant associations in the European American (p=2.36E-15, OR=2.40), African American (p=1.53E-03, OR=2.82,), and US Hispanic (p=8.23E-05, OR 3.52) cohorts. In addition, we identified HLA-DRB1*14:54:01, HLA-DRB1*08:04:01 and HLA-DRB1*16:02:01:02 as additional susceptibility factors in each of these cohorts, respectively. In the Japanese cohort, a completely different haplotype bearing the allele HLA-DRB1*12:01:01:03 showed the strongest association with NMOSD risk (p=3.78E-03, OR 5.71). Additionally, HLA-DPB1*05:01:01 constitutes an independent risk association to NMOSD in both the Japanese (p=6.98E-03, OR 2.13) and the European American (p=1.99E-02, OR 1.84) cohorts; in previous studies this association had been only detected in cohorts with Asian ancestry. Finally, HLA-

DPB1*04:02:01:02 was associated with protection in NMOSD in European Americans, Hispanic Americans, and Japanese cohorts, while the structurally related allele HLA-DPB1*105:01 (with only one amino acid difference at residue 178 in the transmembrane domain) was associated with protection in African Americans. Despite the modest sample sizes, this trans-ancestral study identified main susceptibility and resistance factors that are common across several ancestral groups, alongside novel associations found predominantly in some populations but absent or infrequent in other groups, reflecting broad allelic heterogeneity.

TH44. Spontaneous Negative Selection of Autoreactive T cells in the Human Thymus

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In T-cell mediated autoimmune diseases such as Type 1 Diabetes (T1D), T cells reactive to autoantigens are thought to escape negative selection, traffic to the periphery and attack self-tissues. We aim to determine whether thymic negative selection is impaired in autoimmune-prone human immune systems. We used a T cell receptor (TCR)-transgenic humanized mouse model to study natural autoreactive T-cell development in a fully humanized immune system. Our studies showed that the insulin peptide-specific, HLA-DQ8-restricted Clone 5 TCR, which was isolated from a T1D patient, is negatively selected in healthy human immune systems generated from fetal hematopoietic stem cells (HSCs) and thymus. Evidence included decreased numbers of Clone 5 T cells in the periphery, loss of medullary Clone 5 thymocytes, enrichment of early Clone 5 T cell progenitors that have not undergone thymic selection, and upregulation of negative selection markers on Clone 5 thymocytes. We are currently using this model to study the thymic selection of the Clone 5 TCR in TCR-transgenic mice generated with adult T1D patient and healthy control (HC)-derived HSCs to determine whether these insulin-reactive T cells escape negative selection in T1D immune systems. To our knowledge, these data provide the first evidence of spontaneous negative selection of human autoreactive T cells. Our ongoing studies will determine whether or not negative selection deficiencies contribute to T1D development.

TH46. Predicting seroconversion in first-degree relatives of patients with type 1 diabetes by gene expression analysis

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Type 1 diabetes (T1D) is the most common autoimmune disease in children. While disease development requires a genetic predisposition, less than 10% of genetically-susceptible individuals will develop autoantibodies. There are currently no biomarkers that can predict which individuals will seroconvert. Using peripheral blood RNA samples provided by TrialNet, we performed transcriptome analysis by RNA-Seq to identify early biomarkers of seroconversion in first-degree relatives (FDRs) of T1D patients. We showed that the transcriptome of peripheral blood changes as FDRs get closer to seroconversion. As an example, *FCGR2B* was found to be alternatively spliced in FDRs prior to

seroconversion. *FCGR2B* encodes CD32B, an inhibitory low-affinity Fc-gamma receptor expressed predominantly on B cells that is involved in maintaining immune tolerance and regulating autoantibody production. Loss of CD32B expression leads to a heightened immune response and has been associated with other autoimmune diseases. In FDRs that seroconvert, a marked reduction in the expression of protein-coding isoforms of *FCGR2B* and an increase in non-protein coding isoforms of *FCGR2B* was observed years before seroconversion. In addition, the ratio of protein-coding *FCGR2A* to *FCGR2B* isoforms was also increased prior to seroconversion. *FCGR2A* encodes the activating CD32A receptor on B cells, suggesting that a shift in B cell activity favoring immune activation and autoantibody production occurs prior to seroconversion. The alternative splicing of *FCGR2B* may be a pathogenic upstream event that promotes seroconversion in individuals who are genetically at risk to develop T1D. Thus changes in *FCGR2B* isoform expression could serve as a robust biomarker of seroconversion.

TH57. Single cell analysis of mTECs carrying dominant mutations in functionally distinct domains of AIRE enable a mechanistic understanding of TSA expression

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The autoimmune regulator (AIRE) is a large multi-domain protein that is required for the development of central immune tolerance through its regulation of tissue-specific antigen (TSA) expression in medullary thymic epithelial cells (mTECs). *Aire*-deficiency results in a failure of negative T cell selection and subsequent multiorgan autoimmune disease in both humans and mice. The mechanisms by which the various structural domains of AIRE contribute to TSA expression remain poorly characterized. Recently, a kindred was identified with an autosomal dominant C311Y mutation in the PHD1 zinc finger of AIRE. To better understand PHD1 domain function, we generated a novel knock-in ($Aire^{CY/+}$) mouse model and compared these mice to complete $Aire^{-/-}$ and $Aire^{GW/+}$ mice, which carry a previously described autosomal dominant mutation in the SAND domain. In general, $Aire^{CY/+}$ mice had a unique pattern of affected peripheral organs and a milder blockade in terminal mTEC differentiation. Strikingly, bulk RNA-seq analysis of mTECs revealed that $Aire^{CY/+}$ and $Aire^{GW/+}$ mice shared transcriptomic changes, as expected, but also had significant gene expression changes specific to each mutant domain. These changes included factors involved in DNA binding and chromatin remodeling, implicating both direct and indirect effects of the PHD1 and SAND domains on Aire-mediated gene expression. In sum, our findings elucidate the role of the PHD1 domain in regulating TSA expression and describe a novel mouse model for a human autosomal dominant AIRE mutation.

TH73. Double edged influence of TLR7 and TLR9 on the triggering and severity of lupus

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Toll like receptors (TLR) 7 and 9, endosomal sensors for ssRNA and dsDNA respectively, are important in lupus pathogenesis and could be potential targets for therapy. Although considered homologous with respect to signaling downstream of their respective ligands, they have opposing yet interactive effects on lupus severity. B-cell TLR9 deficiency exacerbates lupus when TLR7 is intact.Our aim is to investigate why TLR7 and TLR9 act differently and if/how they interact in B cells to regulate lupus.

TLR9 could regulate TLR7 by competing for endosomal localization or interaction with MyD88. To differentiate between these hypotheses, a point mutation that blocks TLR9 interaction with MyD88 ($TLR9^{PM}$)was introduced in the endogenous TLR9 locus of MRL/lpr mice using CRISPR/Cas9 targeting.

Ex vivo, $TLR9^{PM}$ is expressed but can't signal upon stimulation.$TLR9^{PM/PM}$ mice had increased survival, decreased immune activation, and ameliorated kidney disease compared to $TLR9^{-/-}$mice. Overall, TLR9 expression, but not TLR9-induced MyD88 signaling, is essential for protection from lupus. However, in B cells, TLR9 didn't directly modulate TLR7 expression nor signaling.

To determine which functional domain(s) of TLR9 are important for protection, we created two types of chimeric TLR7 molecules in TLR9-/- MRL/lpr mice. We replaced the TLR7 ligand-binding or signaling-TIR domains respectively with their TLR9 counterparts. We are now asking which TLR9 domain could restore protection from lupus. These findings will be useful to determine how the TLR pathway could be targeted to efficiently control lupus and will shed light on the basic biology of endosomal TLR signaling.

TH79. Oxidative stress as a therapeutic target in autoimmune myositis: new insights from a murine model.

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Myositides are severe autoimmune diseases leading to disability. A role for oxidative stress has been suggested by the discovery of mitochondrial dysfunctions in dermatomyositis patients. The absence of animal models developing spontaneous myositis has hampered therapeutic research. We genetically invalidated the ICOS signaling pathway on the diabetes-prone NOD mouse background. *Icos*$^{-/-}$ NOD

mice did not develop diabetes, but exhibited spontaneous myositis. The objectives of this study were to investigate the pathogenic role of oxidative stress and to evaluate the therapeutic potential of antioxidant therapy on *Icos*$^{-/-}$ NOD myositis.

Icos$^{-/-}$ NOD mice did not develop diabetes, but developed reduced grip strength and impaired locomotor activity. Myositis was attested histologically by the presence of myofiber necrosis and mononuclear (lymphocyte and macrophage) infiltrates. Elevated muscle free radical production revealed the presence of oxidative stress. Studies of *Icos*$^{-/-}$ NOD muscle proteome by LC-MS/MS (Orbitrap) and oxidative-stress targeted transcriptome revealed the dysregulation of multiple mitochondrial metabolic genes and proteins. Impaired mitochondrial function in *Icos*$^{-/-}$ NOD muscles was supported by disrupted oxidative phosphorylation, evaluated *ex vivo*. Muscle mitochondrial COX, SDH and NADH activities were found to be altered by histoenzymology stainings. Electron microscopy revealed mitochondrial morphological abnormalities in *Icos*$^{-/-}$ NOD muscles. N-acetylcysteine (2 g/L) administration in the drinking water significantly reduced myositis severity. In a curative setting, N-acetylcysteine also ameliorated established disease.

This work demonstrates that Icos pathway disruption shifts auto-immunity from diabetes to myositis in mice and that muscle inflammation is associated to mitochondrial dysfunction and subsequent oxidative stress. It opens new perspectives for therapy.

TH82. Cross-reactive autoimmune CD4+ T cells in narcolepsy

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Narcolepsy is a neurological disorder, caused by selective loss of hypocretin (HCRT)-producing neurons in the lateral hypothalamus. Narcolepsy susceptibility is associated with the HLA-DQA*0102/DQB*0602 (HLA-DQ6) allele and with a SNP in the T cell receptor α chain-encoding gene segment (TRAJ24). We reported the *in vivo* expansion of DQ6/HCRT-specific J24+/CD4+ T cell clones having an unconventional phenotype in patients compared to related clones in DQ6+ healthy controls. Expanded cells show cross-reactive potential as they bind different DQ6/peptide tetramers, suggesting multiple sources of triggers driving CD4+ T cell clonal expansion and potentially mediating autoimmune responses affecting HCRT neurons. Indeed, increased narcolepsy incidence was noted in China after the peak of 2009 H1N1 pandemic influenza (pH1N1) season and in Europe after Pandemrix (pH1N1-derived) vaccine administration. Therefore, we examined CD4+ T cells that are specific for DQ6/HCRT and/or DQ6/HA (pH1N1 hemagglutinin) in our cohort of 12 patients and 12 controls. Given the low frequency in the blood of cells with these specificities, we used DQ6/peptide tetramers to screen >1x10^8 CD4+ T cells to isolate over 10^4 antigen-specific tetramer+ cells for single-cell sequencing. The previously identified, expanded DQ6/HCRT–specific J24+/CD4+ T cell clones are also DQ6/HA-specific, whereas unexpanded DQ6/HCRT-specific clones are not. The specificity and physiologic function of these expanded cells require elucidation. However, our identification of expanded J24+/CD4+ T cell clones exhibiting cross-reactive potential against both HA and HCRT antigens

supports molecular mimicry as a factor in narcolepsy immunopathogenesis and furthers the understanding of autoimmunity in narcolepsy.

TH83. Immunodietica: unveiling the impact of diet in autoimmune disease

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Autoimmune disorders afflict nearly 50 million people in the United States alone. Almost 70% of these cases cannot be explained by genetics alone, suggesting that environmental factors play a key role. Diet is emerging as a risk factor and severity modulator for autoimmunity. Yet, interactions between diet and autoimmunity in humans remain largely unexplored, particularly the impact of immunogenetics, i.e., the human leukocyte antigen (HLA) haplotype of individuals, in this interplay. Here, we systematically interrogated over 20 commonly consumed animals and plants for the presence of epitope sequences implicated in almost 70 human autoimmune diseases, mapping their distribution across organisms, tissue expression pattern, and binding to autoimmune disease-associated and -protective HLA molecules. The species analyzed could be divided into three broad categories based on their human autoimmune epitope content, which we represented using a new metric, the Gershteyn-Ferreira index. Strikingly, pig contains a disproportionately high number of unique autoimmune epitopes not found in other species. Interestingly, in several cases, diet-derived epitopes implicated in a disease are more likely to bind to HLA alleles associated with that disease than to protective HLA alleles. Analysis of an individual's full HLA haplotype allowed for the generation of a personalized heatmap of potential dietary autoimmune triggers, the Gershteyn-Ferreira Sensitivity Passport. Our work uncovers a new link between pork consumption and autoimmune disease in humans and reveals differential binding of diet-derived epitopes to autoimmune disease-associated HLA alleles, laying the foundation for future studies on the impact of diet on the pathogenesis and progression of autoimmunity.

TH91. MicroRNA control of Inflammatory and regulatory T cells in CNS autoimmunity

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Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are mediated by dysregulated autoreactive T cell responses in the central nervous system (CNS). This dysregulation consists of an imbalance between inflammatory T helper cells, such as Th17 cells, and $Foxp3^+$ T regulatory cells (Tregs). MicroRNAs (miRNAs), a class of small non-coding RNAs, are known to play a key role in immune function, and to be dysregulated in EAE and MS. However, identifying specific miRNA pathways that directly connect clinical activity with immune mechanisms in CNS autoimmunity has been a challenge. Previous work has identified miR-92a as one of the most significantly elevated miRNAs in the sera of MS patients, which positively correlates with neurological symptoms and brain atrophy. We now report a major functional role for miR-92a in CNS autoimmunity.

MiR-92a is increased during EAE, and its loss strikingly attenuates clinical disease. This attenuation is accompanied by reduced Th17 and increased Treg cells in the CNS. Mechanistically, we found that T cell-intrinsic miR-92a inhibits the development and function of Treg cells while promoting those of Th17 cells by targeting Foxo1, a key transcription factor implicated in T helper cell differentiation. Preclinical administration of miR-92a inhibitor phenocopies miR-92a loss and ameliorates EAE. Analogous to mice, miR-92a is significantly elevated in MS patient T cells, and reciprocally regulates human Treg and Th17 differentiation. These findings suggest miR-92a skews the Treg/Th17 balance to promote CNS autoimmunity, and that miR-92a silencing may be of therapeutic benefit for MS patients.

TH94. Molecular Profiling of CD25+CD127hi cells points toward a Th-2 based mechanism in prolonging T1D partial remission

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People with Type 1 diabetes (T1D) who undergo partial remission have reduced insulin dependency in the short term and fewer long-term complications. Experiencing a partial remission period is therefore beneficial for people with T1D and studying the mechanisms that promote remission might be helpful in developing strategies that stop and even reverse disease progression. Our research group has identified a novel CD4 T cell population called $CD25^{+}CD127^{hi}$ cells that associates with length of remission (LoR) and response to treatment with Alefacept in people with T1D, with higher frequencies of these cells associating with longer remission. However, how $CD25^{+}CD127^{hi}$ cells might modulate disease progression is still unclear. The purpose of this study was to identify the molecular basis for the $CD25^{+}CD127^{hi}$ cell correlation with disease progression in people with T1D. Protein and transcription factor analyses of $CD25^{+}CD127^{hi}$ cells in healthy individuals indicate a Th2 bias. Differential expression and gene ontology analysis of RNA-Seq data shows significant differences in inflammatory and immune response pathways, apoptotic processes and cell adhesion pathways between the $CD25^{+}CD127^{hi}$ cell population and their closest correlate, $CD25^{-}$ cells. Several Th2-related genes are upregulated including CRTH2 and HPGDS, while Th1-related genes including GZMK and EOMES are downregulated in $CD25^{+}CD127^{hi}$ cells compared to the $CD25^{-}$ cells. Overall, the molecular analyses reinforce a Th2-baised mechanism for the effect of $CD25^{+}CD127^{hi}$ cells in prolonging partial remission and suggest candidates for evaluation as therapeutic targets.

TH95. A Soluble Antigen Array Immunotherapy Intercepts and Prevents the Onset of Type 1 Diabetes in NOD mice

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The efficacy and safety of antigen-specific immunotherapy (ASIT) can be improved by using appropriate delivery platforms that protect and facilitate transport of antigens to lymphoid tissues. We evaluated Soluble Antigen Array (SAgA), a nanoscale platform that bundles multiple copies of peptides from β-cell antigens grafted on hyaluronic acid (HA), in the NOD mouse model of Type 1 diabetes (T1D). SAgAs were prepared in two forms: a hydrolysable form capable of releasing the peptides within endosomes and a non-hydrolysable form that may depend on HA degradation for peptides to be released. Both forms elicited more robust antigen-specific T cell responses in various lymphoid tissues compared to free peptides at equimolar dose. In the treatment of NOD mice at late stage of disease, a mix of two SAgAs carrying a hybrid peptide of insulin and chromogranin A and a mimotope (p79) efficiently prevented the onset of diabetes while each single SAgA failed to provide any significant delay or protection on its own. Repeated treatments improved the therapeutic efficacy, but over time, some mice developed anaphylaxis, which was caused by p79. Anaphylaxis was significantly reduced when peptides were delivered in the hydrolysable SAgA form and nearly abrogated using the non-hydrolysable form. SAgAs induced the upregulation of IL-10 and anergy/exhaustion markers (PD-1+, FR4+ CD73+) in antigen-specific T cells. Interestingly, this tolerogenic phenotype was enhanced with the non-hydrolysable form of SAgA. In sum, SAgAs effectively prevent murine T1D and constitute a novel and promising ASIT platform superior to soluble peptides for the treatment/prevention of T1D.

TH98. Single Cell RNA Sequencing and T Cell Receptor Sequencing Reveal Conserved and Individualized Features of T Cell Responses in Granulomatous Inflammation

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Background

Granulomas occur in multiple human diseases. The pathogenic drivers of non-infectious granulomas, however, are poorly understood. Due to the immunologic restriction of the eye, we hypothesized that studying granulomatous uveitis would provide a robust enrichment of the processes underlying non-infectious granulomas, specifically antigen-specific T cell responses.

Methods

We performed single cell RNA sequencing and T cell receptor (TCR) sequencing of blood and ocular samples from four donors with granulomatous uveitis.

Results

We found that the majority of immune cells in the eye were T cells along with NK cells, myeloid cells, and B cells. CD8 and CD4 T cells had a ratio of approximately 1:2 for all four donors. Ocular CD8 T cells were transcriptionally similar in all donors, showing markers of early differentiation (e.g. CD27 & CD28) and lower expression of cytotoxic molecules GZMB and GNLY. These features paralleled the signature of ocular NK cells. In contrast, CD4 T cells varied across donors, dominated by either TH1/TH17 cells, TH1/GZMB-expressing cells, or Tregs. TCR sequencing revealed highly expanded ocular CD8 and CD4 T cell clonotypes, consistent with antigen-driven responses. While expanded CD8 T cell clonotypes were transcriptionally similar, the signature of expanded CD4 T cell clonotypes reinforced the individual-to-individual variation in granulomatous inflammation.

Conclusion

Our data suggest that lower frequency populations, such as CD8 T cells, display antigen-specific responses that may drive granulomatous uveitis. Additionally, individualized CD4 T cell programs suggest distinct antigen-specific responses. Further investigation of expanded lymphocytes and CD4 T cell phenotypes may identify personalized therapeutic targets.

TH107. ACE Inhibitors Regulate Dendritic Pruning Trough C1q/LAIR-1 in Neuropsychiatric Systemic Lupus Erythematosus Mouse Model

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A frequent manifestation of systemic lupus erythematosus (SLE) is neuropsychiatric lupus (NPSLE). Progressive cognitive impairment occurs in 40-90% of SLE patients, in part through the action of anti-DNA antibodies that cross-react with N-methyl-D-aspartate receptors (NMDAR) which induce neurotoxicity and cell death. Anti-DNA antibodies (DNRAbs), cross-react with the NMDAR and induce NMDAR signaling. In patients, the presence of DNRAb is associated with a spatial memory impairment which is similar to the DNARb$^+$ mouse model.

We have already shown that activated microglia and C1q play a critical role in loss of neuronal dendrites. We have now shown that microglia are the major source of C1q in the CA1 region of the hippocampus using in-situ hybridization. Some studies suggest that Angiotensin-converting enzyme (ACE) inhibitors may suppress microglial activation. Therefore, we aimed to understand how ACE inhibitors protect against or reverse neuronal damage. We showed that captopril could reduce the amount of microglial-produced C1q in DNRAb$^+$ mice. We also demonstrated that LAIR-1 is necessary for the function of ACE inhibitors. Mice treated with captopril exhibited intact dendritic arborization, this did not occur in LAIR-KO mice treated with captopril. Instead, we observed resistant microglial activation despite administration of captopril in LAIR-1 KO mice.

We believe ACE inhibition is a rational potential therapeutic target in clinical trials designed to treat cognitive dysfunction in NPSLE.

TH108. Distinct TCR signaling modules by PRDM1-deficient DCs and the control of follicular helper T cell differentiation

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Genome-wide association study (GWAS) found a polymorphism of *PRDM1* is a risk factor for systemic lupus erythematosus (SLE) and mice with dendritic cells (DCs)-specific deletion of Prdm1 develop lupus-related phenotypes. Only female mice develop disease, suggesting gender dependent mechanisms are critical factors for this disease model. Prdm1 CKO mice have an increased number of T follicular helper cells (T_{FH}) and autoantibodies arising in a germinal center response. In addition, lack of PRDM1 influences on antigen processing through the expression of cathepsin s in DCs, subsequently regulates TCR diversity of T_{FH} cells in spleen. Since the majority of T cell selection and deletion of self-reactive T cells occurs in the thymus, we questioned whether repertoire of thymic T cells is different between Prdm1 CKO and control mice. Thymic DC (tDC) subsets, both resident tDCs and migratory DCs, show no significant difference of thymic antigen presenting cells (APCs) including DCs in *Prdm*1 CKO mice and PRDM1 expression and cathepsin s expression was different in tDCs from Prdm1 CKO mice compared to tDC from control mice. In vitro co-culture of thymocytes (*Nur77*-GFP mice) with thymic DCs isolated from female control or female *Prdm1* CKO mice shows PRDM1-deficient DCs induce a different TCR signal strength. We also compare the TCR repertoire of CD4+ T cells from the thymus of *Prdm1* CKO mice and control mice by high-throughput sequencing analysis. Therefore, we provide insight into how a genetic risk factor, *PRDM1,* alter antigen presentation and contributes to the T_{FH} differentiation and repertoire selection.

TH109. Alteration in metabolic pathway in Tfh cells from SLE patients

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Metabolic changes are closely related to the differentiation and function of immune cells. Different subset of T cells display unique metabolic profiles and inhibition of specific metabolic pathway often leads to altered function or differentiation of T cells. There is a report that CD4+ effector T cells from SLE patients have higher glycolysis compared to CD4+ T cells from healthy individuals, suggesting contribution of altered metabolism to the pathogenic function of lupus T cells. Our study focuses on specific subsets of CD4 T cells, called follicular helper T cells (Tfh: CXCR5+) and peripheral helper T cells (Tph: CXCR5-PD1/ICOS+CXCR3-) mainly due to their cognate function of B cell activation. In this study, we compared metabolism and functional activity of different Tfh and Tph from SLE patients compared to healthy individuals. Functional activity of Tfh and Tph cells was also higher in lupus patients based on their expression of activating molecules and in vitro B cell activation assay. Co-culture of memory B cells with autologous Tfh or Tph cells from SLE patients induces higher level of

plasmablast differentiation and secretion of IgG/ IgA. Interestingly, we found an increased mitochondrial metabolism in Tfh cells (not Tph) from SLE patients than Tfh cells from healthy individuals. However, Tph cells (not Tpfh) from SLE patients have increased induction of glycolysis and mROS production than Tph cells from healthy individuals. These data suggest that different helper T cell subsets from SLE patients have altered metabolic profiles and the alteration is distinct among different effector T subsets.

TH111. Identification of Novel, Clinically Correlated Autoantigens in APS1 and Autoimmune Diabetes by Proteome-wide PhIP-Seq

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The identification of autoantigens remains a critical challenge for understanding and treating autoimmune diseases. Autoimmune polyendocrine syndrome type 1 (APS1), a rare monogenic form of autoimmunity, presents as widespread autoimmunity with T and B cell responses to multiple organs. Importantly, autoantibody discovery in APS1 can illuminate fundamental disease pathogenesis, and many of the antigens found in APS1 extend to more common autoimmune diseases. Here, we performed proteome-wide programmable phage-display (PhIP-Seq) on sera from a cohort of people with APS1 and discovered multiple common antibody targets. These novel APS1 autoantigens exhibit tissue-restricted expression, including expression in enteroendocrine cells, pineal gland, and dental enamel. Using detailed clinical phenotyping, we find novel associations between autoantibodies and organ-restricted autoimmunity, including a link between anti-KHDC3L autoantibodies and premature ovarian insufficiency, and between anti-RFX6 autoantibodies and diarrheal-type intestinal dysfunction. In addition, we employ PhIP-Seq to probe autoantibody-negative type 1 diabetes and checkpoint inhibitor-induced autoimmune diabetes for novel, diabetes-associated candidate autoantigens. These studies highlight the utility of PhIP-Seq for extensively interrogating antigenic repertoires in human autoimmunity and the importance of antigen discovery for improved understanding of disease mechanisms.

TH117. Integrating multi-omic and clinical information using innovative statistical approaches to deliver precision medicine.

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To facilitate delivering precision medicine in immune-mediated inflammatory diseases (IMIDs) studies have focused on identifying clinically relevant biomarkers to predict diagnosis and prognosis. Despite

these efforts, few studies have been successful. This could be driven by several confounding factors such as heterogeneity among the patients and/or the biological specimens collected. Our current research aims to build on and extend an existing programme that has delivered a prognostic biomarker test into clinical practice, by establishing a cohort of clinically active patients across a range of IMIDs and extending the analysis to examine different immune celltypes.

We enrolled 149 largely treatment-naive patients with Crohn's disease, ulcerative colitis, ANCA-associated vasculitis, systemic lupus erythematosus, or idiopathic pulmonary fibrosis, and 60 matched healthy volunteers and followed them over time. Serum, whole blood, and PBMCs were collected from each individual at enrolment. To better understand the contribution of different immune cells to disease onset and pathogenesis, we designed two 15-colour antibody panels enabling us to sort by flow cytometry up to 16 different leukocyte cells covering the innate and adaptive immune systems. Total RNA, small RNA, and gDNA molecules were extracted from all the collected samples and used to generate cell specific mRNA and miRNA transcriptomes, and DNA methylomes respectively.

We have established a unique multi-omic resource derived from patients with active IMIDs recruited before treatment. Innovative and integrative analyses of these new omic datasets and clinical data will shed light on our understanding of, and enhance our ability to manage, these important IMIDs.

TH120. Adaptive Design of a Phase 2, Placebo-Controlled, Dose-Ranging Study to Assess the Efficacy and Safety of Rozibafusp Alfa in Subjects With Active SLE and Inadequate Response to Standard of Care Therapy

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Background Barriers to drug development in systemic lupus erythematosus (SLE) include disease heterogeneity, lack of outcome measures, and substantial placebo response rates. Optimized trial design is important for an objective evaluation of clinical response. Rozibafusp alfa (AMG 570) is a bispecific antibody-peptide conjugate that inhibits both ICOSL and BAFF and reduces circulating naïve B cells in healthy subjects. This phase 2 study will employ SLE drug screening and innovative response-adaptive randomization (RAR) to optimize dose selection in subjects with active SLE and inadequate response to standard of care (SOC) therapy (NCT04058028).

Methods Subjects (N~300, age 18–75 years) will be randomized to receive placebo or 1 of 3 doses of rozibafusp alfa subcutaneously Q2W for 52 weeks, with 16 weeks of follow-up. The primary objective is to evaluate efficacy of rozibafusp alfa vs placebo at week 24 using the SLE Responder Index (SRI-4). Screening visits will fulfill criteria for active SLE and demonstrate stability of concomitant therapy, including OCS, immunosuppressants, and/or immunomodulators. SOC drug screening will confirm serum drug levels at baseline (and hence treatment-refractory status), which may help control the placebo response rate. RAR aims to allocate more subjects to more efficacious doses while maintaining the placebo allocation constant; the randomization ratio could be adapted after interim analyses based on clinical efficacy.

Results Study ongoing.

Conclusion This study will provide safety and efficacy data for rozibafusp alfa compared with placebo, and its adaptive design aims to optimize development of a novel therapy for SLE patients with inadequate response to SOC.

TH131. A Gain-Of-Function Mutation in the SKAP2 Integrin Signaling Adaptor Protein Causes Autoimmune Diabetes

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Autoimmune diabetes is the most common form of diabetes in children. Whole exome sequencing in a patient with a variety of autoimmune pathologies, including autoimmune diabetes, identified a *de novo* heterozygous c.457G >A/p.Gly153Arg mutation in the Src kinase-associated phosphoprotein 2 (SKAP2) gene, a critical component of the integrin-mediated “outside-in” signaling pathway in myeloid immune cells. We show that integrin activation in patient-derived macrophages and neutrophils resulted in enhanced physiological functions. Integrin activation of patient-derived neutrophils resulted in enhanced release of reactive oxygen species while patient-derived macrophages displayed a migratory phenotype in the absence of chemokine stimulation. The p.Gly153Arg variant, located in a well-conserved ‘lipid-gated switch domain’, induced similar phenotypes when expressed in a human macrophage cell line. Our data indicate that SKAP2 mutations cause hyperactivation of integrin-mediated outside-in signaling pathways in myeloid cells, resulting in human autoimmune diabetes.

TH134. Beneficial Effects of BCG Vaccinations in Long-Term Type 1 Diabetes: Updated Clinical Trial Data and Epigenetic Impact on Foxp3 Treg Methylation

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Global human clinical trial data shows that the bacillus Calmette-Guerin (BCG) vaccine has broad heterologous benefits. We are evaluating BCG for its potential as a safe and affordable vaccine for individuals with established (>10 y) type 1 diabetes (T1D), focusing in open-label and blinded clinical trials on restoring Treg balance and correcting underlying glucose utilization defects. Here we present updated, open-label clinical trial data in adult long-term T1D cohorts receiving 2 BCG vaccines and followed for > 1 year (n=6) with a clinical phenotype of juvenile onset diabetes of long duration (median

age at onset 11+/-6 y, duration 18 +/-6 y, current age 29+/- 2 y). The open-label BCG multi-dosed subjects demonstrated consistent lowering of HbA1c values by over 6% at the one-year time point. Both *in vivo* and *in vitro* data show the lymphoid system improves aerobic glycolysis, and thus accelerates sugar uptake, after BCG. BCG also epigenetically modified the Foxp3 Treg signature gene by demethylation through year 01. Improved metabolic sugar utilization in monocytes after BCG in long term T1D with early onset disease improves blood sugar regulation with lowering of HbA1c. On the immune side, BCG also starts to demethylate 15 cg sites of the Foxp3 Treg gene to restore suppressor cells. This open-label data documents the reproducible influence of repeat BCG vaccination on significant HbA1c lowering in early onset T1D with long-term disease.

TH135. A new regulatory cell in autoimmunity: Human KIR+ CD8+ T cells target autoreactive cells

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$CD8^+$T cells are well known for their ability to kill cells harboring pathogens, but a subset of $CD8^+$T cells expressing Ly49 proteins in mice have been reported to have a regulatory function. Stimulating this subset with surrogate peptides can suppress self-reactive MOG-specific $CD4^+$T cells and thereby ameliorate mouse experimental autoimmune encephalomyelitis. However, it is yet to be determined whether a similar population of regulatory $CD8^+$T cells exists in humans. Here we show an increased frequency of peripheral $CD8^+$T cells expressing Killer cell Immunoglobulin like Receptor genes (KIR), the evolutionary counterpart of mouse Ly49, in a subset of patients with different autoimmune diseases. Several lines of evidence suggest KIR^+CD8^+ T cell is the functional and phenotypic equivalent of mouse $Ly49^+$ $CD8^+$T cells, and likely suppress autoreactive $CD4^+$T cells via cytotoxicity. Expanded KIR^+CD8^+T cells have common features shared by healthy subjects and different autoimmune diseases, yet also display some heterogeneity associated with different diseases or treatments. Moreover, KIR^+CD8^+ T cells are found in inflamed tissues and are phenotypically similar to those in the blood. Overall, we define and characterize a new regulatory $CD8^+$T cell subset in humans, with important implications for the cellular dynamics and possible therapeutic approaches to human autoimmune diseases.

TH282. The concept of immune system reboot therapy (ISRT) in autoimmune diseases

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Autoimmune diseases as a collective are generally secondary to a dysregulation of the innate and/or adaptive immune systems leading to organ and/or systemic disease in a genetically and environmentally predisposed host. There is no cure for autoimmune diseases currently, but therapy consists of controlling the chronic systemic disease towards remission, supporting affected organs and preventing acute flares using corticosteroids, disease modifying anti-rheumatic drugs (DMARDs) and more recently biologic therapies.

An ideal cure would consist of wiping out the existing immune system and replacing it with an alternative (stem cell bone marrow transplant) which is neither a safe nor easy option to acquire. An alternative would be rebooting the existing immune system. The latter would consist of eliminating all existing immune system memory, and allowing the pluripotent stem cells of the host to reboot (ISRT), without reproducing the disease mimicking the original stem cell at birth since autoimmune disease are not congenital. The objective of this review is to look into literature for evidence supporting a succesful realisation of ISRT and its potential to cure autoimmune diseases.

Prolonged supervised fasting is one of the potential treatment modalities to be explored since it deprives rapidly diving cells such as the immune cells including the bone marrow of cell division precursors, leading to inhibition of DNA replication, cell division and clearance of compromised immune cells, in this manner rebooting the compromised bone marrow and immune system in its entirety.

TH283. Cystatin C Contributes To Sex Difference In Experimental Allergic Encephalomyelitis

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In multiple sclerosis (MS), 2-4 times more women develop the disease compared to men. Sex chromosomes and sex hormones have been implicated but the molecular factors mediating their actions are largely unknown. Towards clarifying the role of the cysteine protease inhibitor, Cystatin C (CysC), whose expression is augmented in the brains of MS patients and mice with experimental allergic encephalomyelitis (EAE - a model of MS), we discovered that CysC plays a detrimental function in EAE, but only in female animals. Female CysC null mice displayed significantly lower clinical signs of disease compared to wildtype (WT) littermates that was associated with reduced activation and antigen presenting ability of antigen presenting cells (APCs) such as macrophages. No differences in EAE signs or APC function were noted between male WT and $CysC^{-/-}$ mice and cells. This detrimental function of CysC in female macrophages and EAE was due to CysC interacting with female sex hormones because ovariectomy + testosterone administration in female WT and $CysC^{-/-}$ EAE mice abolished the clinical difference between these two genotypes while male CysC null mice that normally show no difference in EAE signs relative to their WT counterparts, displayed marked reduction in disability upon castration and estrogen/progesterone administration. Altogether, we discovered that a collusion between Cystatin C and female sex hormones in APCs such as macrophages may partly explain why women have a higher incidence of MS compared to men. *Funded by The Canadian Institutes of Health Research and the MS Society of Canada.*

TH285. Langerin-expressing Dermal Dendritic Cells and Epidermal Langerhans Cells Elicit Paradoxical, Balancing Roles in the Pathogenesis of Autoimmune Skin Inflammation

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Skin harbors two subsets of langerin-expressing dendritic cells (DC), namely Langerhans cells (LC) in the epidermis, and langerin-expressing dermal DC (LangdDC) in the dermis. We previously reported that the migration of LC is impaired in the MRL model of autoimmune dermatitis (Eriksson and Singh, J Immunol 2008), and this defective migration plays a role in the loss of tolerance to skin antigens (King J…Singh RR, J Immunol 2015). Here, we investigated roles of the two langerin-expressing DC subsets, LC and LangdDC, in the pathogenesis of autoimmune skin inflammation. To track langerin-expressing DCs, we used langerin-eGFP gene knock-in mice in the autoimmune (MRL-lpr and MRL+/+) and healthy control (C57Bl/6) backgrounds. We found a reduced migration of LC, but increased trafficking of LangdDC, to skin-draining lymph nodes in autoimmune mice as compared to healthy controls. Such altered pattern of migration of LC and LangdDC was corrected by αGalCer treatment that ameliorates autoimmune dermatitis. Finally, conditional ablation of LC worsened autoimmune dermatitis; this effect was abrogated when both LC and LangdDC were ablated together. LC depletion did not affect kidney or lung disease. In summary, LC and LangdDC play opposite, balancing roles in the pathogenesis of autoimmune dermatitis: LC protect and LangdDC harm. The two skin DCs are also regulated differently: LC migrate less, but LangdDC traffic more, to skin-draining lymph nodes of autoimmune mice. Such tight regulation of immunity/autoimmunity at the tissue level can be targeted to control inflammation in an organ-specific manner, thus reducing the risk of systemic immune-suppression.

TH286. Mortality Burden of Immune-Mediated Inflammatory Diseases (IMID) in the United States

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Primary immune-mediated diseases, collectively referred to as immune-mediated inflammatory diseases (IMID), involve all organs, affect >23.5-million Americans, and cost >100-billion dollars in the US. However, their collective burden of disease is unknown. Here, we determined the burden of IMID deaths relative to the CDC's leading-causes-of death ranking, and assessed demographic/geographic determinants of IMID mortality.

Methods: We used the CDC-WONDER database to obtain death counts of 43 IMIDs with a prevalence of >1/100,000 population, and calculated their death rates. We then ranked the pooled death counts of the 15 IMIDs with the highest death rates among the CDC's official leading-causes-of-death ranklist. Next, we performed multinomial regression analysis to determine associations of IMID mortality with sex, race, sex, age, and residence.

Results: 371,154 deaths were attributed to the top 15 IMIDs from 2013-2017. IMID deaths ranked 6th-9th among the CDC's leading-causes-of-death ranklist. Females, black persons, and residents of the West had higher premature mortality for IMID relative to the general population. American Indian/Alaska

Native residents of the Midwest and West exhibited the highest IMID mortality risk relative to general population.

Conclusions: Ranking of IMID among the leading causes of death emphasizes their high burden of disease. Akin to cancers, IMIDs should be considered as a collective disease entity. The recognition of IMID as a major public health problem may influence healthcare prioritization, and research funding, which is ~7-fold less for IMID than for cancers (965-million vs 6,610-million US dollars in 2019). Furthermore, profound sex, race and geographic disparities exist in IMID mortality.

TH287. Identification of Cell-Surface Markers Associated with Regulatory T Cells in Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune disease associated with altered regulatory T cell (Treg) suppressive function. We selected nine proteins associated with Treg inhibitory capacity and investigated whether their expression was altered in MS patients.

PBMCs were obtained from untreated MS patients and from age and sex-matched controls (n = 31). Multi-color flow cytometry was used to measure the markers in T $CD4^+$ $Foxp3^-$ cells, Tregs, $Helios^+$ Tregs (nTregs), and $Helios^-$ Tregs (pTregs).

MS patients had an increase in the percentage of pTregs (all, p = 0.0237; progressive, p = 0.0429). There was an increase in the percentage of $BTLA^+$ Tregs in all MS (p = 0.0446) and in RRMS patients (p = 0.0250), an increase in the percentage of $GARP^+$ Tregs in all MS (p = 0.0357) and in RRMS patients (p = 0.0261), an increase in $DNAM1^+$ Tregs in P-MS patients (p = 0.0315), and a decrease in $ABCA1^+$ Tregs in all MS patients (p = 0.0449). There was a positive correlation between $ABCA1^+$ Tregs and the age of disease onset (r = 0.54, p = 0.04), and negative correlations between $ABCA1^+$ Tregs and EDSS score (r = -0.76, p = 0.0009) and between $ABCA1^+$ Tregs and EDSS/years (r = -0.64, p = 0.0097).

Our results show altered frequencies of previously unknown surface markers in Tregs from MS patients that could contribute to their altered functional capacity.

TH292. The role of CD8:B-lymphocyte interactions in type 1 diabetes pathogenesis

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Human leukocyte antigen (HLA) class I molecules on antigen presenting cells (APCs) cross-present peptides derived from extracellular proteins to $CD8^+$ T cells, to induce cytotoxic T cell responses (CTL).This interaction may be important in Type 1 Diabetes (T1D) where autoreactive $CD8^+$ T cells interact with self-peptide-HLA-I complexes to mediate β-cell damage. The key APCs mediating this process remain elusive, however, since recent evidence has highlighted a role for B cell involvement in T1D, we hypothesise that B cells cross-present antigen to $CD8^+$ T cells and maintain CTL relevant to β-cell destruction. Using an antigen delivery system (ADS) to target antigen uptake via the B cell receptor (BCR), co-culture of ADS-pulsed B cells with $CD8^+$ T cell clones induced T cell degranulation. Within these co-cultures, CD20 and CD8 positive doublets with high CD107α expression were identified in a peptide dependent manner and visualised using imaging flow cytometry, suggesting interacting $CD8^+$ T cells and $CD20^+$ B cells. As a comparison of cross-presentation potency, immature monocyte-derived dendritic cells were matured with the same antigen and showed comparable degranulation. Identification by immunofluorescence microscopy of B cells within the pancreatic tissue of T1D patients, some in association with CD8+ T cells, further supports their role in disease and laser-capture microdissection of these B cells will allow molecular insight into their IGHV repertoire. Thus, these data suggest that B cells can cross-present antigen and induce CTL; a feature that may be relevant to their role in the cellular and molecular interactions that underlie T1D tissue pathology

TH327. Metabolic Regulation of Type 3 Innate Lymphoid Cells by Intestinal Bacteria-Derived Indoles in Ankylosing Spondylitis

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Background: Intestinal microbial dysbiosis, intestinal inflammation, and Th17 immunity are all linked to the pathophysiology of ankylosing spondylitis (AS); however, the mechanisms linking them remain unknown. Using three unbiased approaches, we hypothesized that microbial dysbiosis in AS results in altered bacterial metabolites that expand IL-17-producing immune cells.

Methods: Healthy controls (HC, N=24) and patients with AS (N=23) were recruited while undergoing standard-of-care colonoscopy. Thirty distal colon biopsies and rectal swabs were obtained from each subject. Four biopsies were submitted for metabolomics screening via LC-MS while the remaining were homogenized and analyzed by single cell RNA sequencing (scRNA-seq, 2 subjects per group) and flow cytometry (remaining) to validate findings. Bacterial DNA was extracted from the rectal swabs, underwent shotgun sequencing, and analyzed using the HumanN2 software suite.

Results: ScRNA-seq identified a unique population in AS versus HC that transcriptionally segregated with T cells but lacked traditional T cell markers. Flow cytometry validated a significant expansion of type 3 innate lymphoid cells (ILC3s) in the intestinal tissue from patients with AS. By LC-MS, multiple metabolites within the tryptophan pathway were found to be significantly increased in AS, and

metagenomic analysis of the bacterial population in AS identified altered metabolic pathways involving amino acid synthesis/degradation, particularly tryptophan.

Conclusions: In this study we identified altered tryptophan metabolism with a corresponding increase in ILC3s in intestinal tissue from patients with AS, which may link intestinal pathology to Th17 immunity in AS. Future studies will focus on the mechanistic pathways between tryptophan metabolism and ILC3 development.

TH344. CD8 T cell exhaustion, increased CD4+CD8+ T-cells and aberrant cytokines in Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS).

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a complex disorder affecting numerous organ systems and biological processes. Published data suggest that ME/CFS may be preceded by infection, and the chronic manifestation of illness may represent an altered host response to infection, or an inability to resolve inflammation. Here, we hypothesize that in ME/CFS an aberrant response to an immunological trigger like infection may result in a dysregulated immune system, leading to a immunosuppression. We examined CD8+ and CD4+CD8+ T-cells to determine whether their frequency and cytokine production was altered in ME/CFS patients as compared to healthy donors. We analyzed the T-cell receptor (TCR) repertoire of both populations looking for evidence consistent with a viral or auto-antigen driven response. We observed altered expression of exhaustion markers like CTLA4 and 2B4, decrease in CD8 T-cell number, and function, particularly CD107ab and IFNg production. This was associated with a compensatory increased frequency of activated CD4+CD8+ T cells in ME/CFS patients. Both T cell populations were spontaneously producing cytokines, subdividing into two types of ME/CFS: (1) FoxP3+ cells producing IL9 (female donors), (2) IL17-producing cells (male donors). These results are consistent with immunosuppression mediated via exhaustion of CD8 T-cells as observed either in chronic viral infections or tumor environments. The observed exhaustion was associated with a compensatory increase in activated CD4+CD8+ that make unusual cytokines known to interact with the nervous system. These findings identify potential biomarkers and mechanisms driving the immunopathogenesis of ME/CFS leading to future therapies (Funding: Ramsay Award, Solve ME/CFS Initiative).

TH348. Applying Machine Learning Approaches to Synovium and Blood Gene Expression Data Uncovers Novel Biomarkers for Rheumatoid Arthritis

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There is an urgent need to develop objective biomarkers for early diagnosis and monitoring of disease activity in Rheumatoid arthritis (RA). We defined a RA meta-profile using publicly available cross-tissue

gene expression data and applied machine learning to identify putative biomarkers, which we further validate in single cell data.

Raw data from 284 synovium (SY) and 1,885 whole blood (WB) microarray samples was downloaded and processed. A robust feature selection pipeline was applied it to identify a set of genes in both tissues in training data. Using a performance threshold AUC ³ 0.7 in three independent validation testing datasets from synovium and PBMCs, 14 genes were identified as biomarkers: 12 up-regulated (*TNFAIP6, S100A8, TNFSF10, DRAM1, GPR65, LY96, SAMSN1, KYNU, SQOR, ENTPD1, CLIC1, ATP6V0E1*) and 2 down-regulated (*TNPO2, CIRBP*), whose expressions correlated with clinical measures individually and as a joint "RA score". Four proteins, TNFSF10, TNFAIP6, S100A8, and LY96, are secreted in blood based on secretome datasets. For further validation and investigation of biological pathways involved, we generated single-cell RNA-seq data from PBMC of 23 untreated RA patients and 23 matched healthy individuals. We found these genes to be more expressed in monocytes, B-cells, CD4+ and CD8+ T-cells among RA patients.

This novel list of biomarkers, identified through a feature selection procedure and validated using multiple data types, may be useful in the early diagnosis and disease monitoring of RA.

TH354. T cell Recognition of minor HLA DR associated antigens in subjects with Type 1 Diabetes

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Type 1 diabetes (T1D) is caused by the immune mediated destruction of pancreatic beta cells. Autoantibodies and autoreactive T cells that recognize multiple antigens accumulate leading up to disease onset. A recent study explored a number of novel autoantigens in patients with type 1 diabetes, documenting antibodies to NUP50 that were associated with HLA-DRB1*03 and antibodies to MLH1 that were associated with HLA-DRB1*04 genotypes. In light of this, we investigated the recognition of these antigens by CD4+ T cells. Utilizing in vitro binding assays we identified peptides derived from NUP50 and MLH1 capable of competing for binding to DRB1*03:01 and DRB1*04:01 and forming stable HLA class II tetramers. Utilizing an *in vitro* tetramer-based assay, we then verified that a subset of those peptides are capable of eliciting T cell responses in the peripheral blood of subjects with these HLA haplotypes. We then performed direct *ex vivo* staining of peripheral blood to demonstrate that memory T cells that recognize these peptides are present in subjects with T1D. These findings demonstrate that HLA-associated responses two novel antigens (NUP50 and MLH1) are present in subjects with T1D and suggest that monitoring antibody and T cell responses to these antigens could provide important new insights about disease progression.

TH357. Gene Expression Profiling of Discoid Lupus Erythematosus in Dogs, Humans, and Mice Reveals Conserved Inflammatory and Skin Specific Gene Signatures

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Autoimmune skin diseases are complex in nature and are thought to arise from a combination of genetics and environmental exposures, which trigger an ongoing immune response against self. Companion animals including cats and dogs are known to develop inflammatory skin conditions similar to humans, providing opportunities to study spontaneous disease from biopsies taken for diagnostic purposes. A strength of comparative immunology approaches is that immune profiles may be assessed across different species to better identify shared or conserved pathways that might drive inflammation. Here, we performed a comparative study of skin from two different canine cutaneous lupus erythematosus (CLE) subtypes, discoid (DLE) and mucocutaneous (MLE), using Nanostring nCounter technology. We compared these gene expression patterns to those of human DLE and a mouse model of DLE. We present data indicating shared inflammatory signatures exist across species, underscoring these as potential drivers of DLE.

TH359. Failure of B cell Tolerance is Associated with Increased Peripheral Helper T cells in "Personalized Immune" Mice Generated with Hematopoietic Stem Cells from Patients with Type 1 Diabetes

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Interactions between B cells and $CD4^+$ T helper cells play a central role in the development of Type 1 Diabetes (T1D), by promoting the production of islet-specific autoantibodies. Recent studies have shown two different B-cell-helper T cell subsets, including follicular (Tfh) and peripheral (Tph) helper T cells, to be increased in patients with T1D. It is not known whether Tfh and Tph cells play a role in the development of B cell abnormalities and loss of B cell tolerance in T1D. To address these questions, we used a personalized immune (PI) humanized mouse model where we generated a *de novo* immune system from hematopoietic stem cells (HSC) of patients with T1D or from healthy controls (HCs). We observed that mice injected with T1D HSC had increased percentages of Tph but not Tfh cells compared to HC-derived PI mice. Consistently, we demonstrated several abnormalities in the distribution of B cell subsets, including elevated proportions of unconventional memory $CD27^-IgD^-$ B cells and $CD27^{high}CD38^{high}CD138^-$ plasmablasts, but reduced proportions of naive $CD27^-IgD^+$ B cells and $CD27^{high}CD38^{high}CD138^+$ plasmacells in T1D- compared to HC-derived PI mice. Moreover, these changes in B cell subsets correlated with the levels of Tfh and Tph cells. Our findings suggest that T1D HSCs are genetically programmed to produce increased proportions of Tph and possibly Tfh cells and that these may promote the development of unconventional and autoreactive B cells. These findings in PI mice provide an avenue for further understanding of the immune abnormalities that drive T1D.

TH360. Distinct Exhausted CD8 Subsets Linked to Preservation of C-peptide in Alefacept-Treated, Recent Onset Type 1 Diabetes (T1D) Subjects

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Trials of biologic therapies in type 1 diabetes (T1D) aim to mitigate autoimmune destruction of pancreatic beta cells and represent a resource of immune perturbations to elucidate mechanisms important in disease and therapy. In the T1DAL trial of alefacept (LFA3-Ig, a soluble ligand for CD2) in recent onset T1D, endogenous insulin production was preserved in 30% of subjects for two years after therapy. In light of our previous findings linking exhausted CD8 T cells to beneficial response in T1D trials, we applied a set of unbiased analyses to sorted CD8 T cells to evaluate their potential role in T1DAL. Using RNA-seq, we found that greater insulin C-peptide preservation was associated with a module of activation and exhaustion-associated genes. This gene signature was further dissected into two distinct CD8 effector memory populations through correlation with clustered cytometry data. Both populations were hypo-proliferative in vitro and shared expression of multiple markers characteristic of exhausted cells (TIGIT, KLRG1, TBX21, EOMES, etc. The populations were distinguished by reciprocal expression of human CD8 T and NK cell genes (GZMB, CD57 and iKIR genes), versus PDCD1 and T cell activation and differentiation genes (IL-2, CD28). Further analysis showed these to be subsets that shared expanded TCR junctions, suggesting a clonal relationship. These findings support previous evidence linking exhausted CD8 T cells to successful immune interventions for T1D, while also suggesting that multiple inhibitory mechanisms are involved in promoting this beneficial cell state.

TH362. Differential impacts of TNFa inhibitors on the expression of Th cytokines

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Inhibition of TNFa has emerged as an effective therapeutic approach for many autoimmune/inflammatory diseases. While the efficacy of the FDA-approved TNFa inhibitors (TNFis), including etanercept, adalimumab, certolizumab, golimumab, and infliximab, in rheumatoid arthritis (RA) is comparable, there exist some intriguing differences among them. For example, one RA patient may respond to one TNFi but not the others; etanercept is generally ineffective for inflammatory bowel diseases or uveitis. Post-hoc data have also uncovered several unexpected sided effects of TNFis, such as +ANA, lupus-like diseases, demyelinating diseases, and pustular psoriasis, which can be partly attributed to the induction of type 1 interferons. Interestingly, certolizumab may be less likely to induce lupus-like diseases. The mechanisms are still poorly understood.

Here we report that adalimumab and etanercept, but not certolizumab, inhibited the expression of IL-17A/F and IL-2 in Th cells within anti-CD3-stimulated PBMC. This discordant effect between adalimumab and certolizumab was not due to differential neutralization of soluble TNFa or binding to transmembrane TNFa. Instead, the unique effect of adalimumab required cell-cell contacts between Th cells and non-Th cells. RNA-seq analyses further revealed that adalimumab, but not certolizumab, also

suppressed the expression of other effector Th cytokines, including IFNg, IL-21, and IL-9, but reciprocally induced strong type 1 interferon signals and the expression of co-inhibitory molecules CD96 and PVRIG in Th cells. The cause is still being investigated. Elucidating the mechanism mediating the discordance between adalimumab and certolizumab very likely will uncover novel mechanisms of action of TNFis and improve their efficacy and safety.

TH371. Resistance of autoimmune T cells to TGFβ-mediated regulation:a novel biomarker and therapeutic target for type 1 diabetes

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Type 1 diabetes (T1D) results from the destruction of insulin-producing pancreatic β cells by autoreactive T cells. We have documented that the frequency of circulating autoreactive T cells were similar in healthy individuals and T1D patients, arguing for the existence of a universal 'benign' autoimmunity. Progression to T1D may rely on a loss of immune regulation that controls autoreactive T cells, which may be cell-extrinsic but also cell-intrinsic. Indeed, our preliminary findings in the non-obese diabetic (NOD) mouse model of T1D document a progressive T-cell refractoriness to TGF-β-mediated suppression. We are here investigating whether a defective TGF-β signaling drives autoimmune T-cell escape from regulation and can be used as a biomarker of progressive β-cell autoimmunity and as a therapeutic target.

We first analyzed the expression of main canonical TGF-β pathway activatory (Smad2/3) and inhibitory (Smad7) members in T cells from NOD mice and control strains. Smad7 was upregulated in T cells from NOD mice, notably in the effector subset infiltrating the pancreas. This was paralleled with an impaired Smad2/3 phosphorylation upon TGF-β stimulation. This altered signal translated into a poor inhibition of NOD T-cell proliferation and a reduced capacity of $CD4^+$ conventional T cells (Tconv) to convert into $Foxp3^+$ regulatory T cells and to upregulate PD-1 in response to TGF-β. Blockade of Smad7 expression allowed Tconv from diabetic NOD mice to recover sensitivity to TGF-β (proliferation assay). Altogether, these data pinpoint to an intrinsically defective TGF-β pathway, contributing to an altered induction of regulatory T-cell responses in NOD mice.

TH388. CXCR3 blockade reduces germinal centers and autoantibody titers, but not skin disease, in murine cutaneous lupus erythematosus

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Cutaneous Lupus Erythematosus (CLE) describes a broad range of autoimmune dermatologic diseases that are characterized histopathologically by interface dermatitis and autoantibody deposition. Understanding the pathogenesis of CLE is an important step in developing effective treatments for patients. One of the most highly upregulated chemokine families in CLE is the CXCR3 chemokine family. The interaction between CXCR3-expressing T-cells and its ligands have been associated with tissue damage in CLE subtypes. To further understand the role of CXCR3 in CLE immunopathogenesis, we performed studies using a mouse model of CLE and human tissue. Here, we characterize CXCR3-bearing immune cells in the skin of this mouse model and in blister biopsies obtained from CLE patients. We also wanted to determine whether CXCR3 blockade with a monoclonal antibody could prevent CLE disease development. We show that CXCR3 blockade in CLE mouse models did not significantly reduce skin disease, but helped reduce autoantibody titers and germinal centers. The significance of our findings is twofold. First, out data suggest that blockade of CXCR3 may have prevented the stimulation of autoantibody-secreting B cells, resulting in reduced autoantibody titers. This supports the previously published role of TBX21/Tbet in B cell responses in a model of SLE. Our results also suggest the presence of an alternative CXCR3-independent pathway that is responsible for tissue recruitment to the skin in CLE, providing an explanation for why CLE can exist as an independent entity without systemic involvement. Together these data contribute to our understanding of splenic versus skin tissue responses in lupus.

TH392. Cold Exposure Protects from Neuroinflammation through Immunologic Reprogramming

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Obesity is linked to development of metabolic and inflammatory diseases. However, effects of a negative energy balance and a metabolically active phenotype on the immune system and immune-mediated diseases are poorly understood.

Here we use cold exposure as an inducer of energy expenditure, which mainly acts by activating the UCP1-mediated brown adipose tissue thermogenesis. We show that cold exposure modulates monocytes and consequently T cell priming, resulting in decreased disease severity in a mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Specifically, we found that cold exposure reduces monocytes in the bone marrow and changes their immunologic and metabolic phenotype in the circulation. Exposure to cold temperatures decreases the EAE severity independent of UCP1-mediated thermogenesis. Cold exposure reduces pathogenic T cell cytokine expression and MHCII expression of monocytes during EAE. Depleting the monocytes via genetic or pharmacological CCR2 blockade abolished T cell cytokine expression at EAE onset, implying that cold exposure may affect T cell priming via modulation of monocytes. Accordingly, EAE is unchanged when cold exposure is applied only during the effector phase of the disease.

Our work provides systematic overview on the immune changes during exposure to cold and could have implications in prevention and treatment of immune-mediated diseases.

TH397. Identification of Novel and Repurposing Drugs for the Reduction of IL-1β and TNF-α Secretion by Inflamed Cells: Proof of Concept in Rheumatoid Arthritis

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Rheumatoid Arthritis (RA) is an autoimmune chronic disease, characterized by inflammation in multiple joints, ultimately leading to joint deformity, pain and swelling. A high percentage of patients do not respond to currently available therapies. Our research aims to identify novel immunotherapeutics for RA, using a drug-centric, rather than a target-centric, approach. First, we performed an *in silico* screening of our extensive chemical library (60,000 compounds, containing repurposing drugs, targeted libraries and lead-like compounds) to select representative compounds of the whole chemical space (n=3,880). Next, we developed an *in vitro* inflammation model with human peripheral blood mononuclear cells (PBMCs), and through a biophysical high-throughput assay we selected representatives capable of binding the cells. We then selected those compounds capable of reducing the secretion of TNF-α, IL-1β or both, while do not altering cell viability (n=20), through multiplex cytokine measurement and viability assays. Of those 20 candidates, we selected compounds following Lipinski rules and/or being repurposing drugs, and we validated them in different donors and at different concentrations. The extensive screening effort led to 3 strong candidates (two repurposing drugs and one novel molecule), which allow for 30-50% reduction of IL-1β and/or TNF-α secretion by inflamed PBMCs. Further, we validated this reduction in cytokine secretion in PBMCs from newly diagnosed RA patients which have not yet received any treatment. In summary, we identified novel compounds capable of reducing IL-1β and TNF-α secretion in both *in vitro* inflamed HD PBMCs and PBMCs from naïve RA patients

TH403. The Role of Retrotransposon LINE-1 in Inflammatory Diseases and the Potential Therapeutic Modulation by an Anti-HIV-1 Nucleoside Reverse Transcriptase Inhibitor (NRTI) Emtricitabine (FTC)

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Introduction Type I interferon (IFN-I) is a central mediator in many inflammatory and autoimmune diseases such as lupus. It is hypothesized that aberrant nucleic acids present in lupus patients may trigger the innate immune response and induces IFN-I. One possible source of such aberrant nucleic acids is the Long Interspersed Nuclear Element-1 (LINE-1) retrotransposon that comprises 17% of the human genome and is normally silent. Dysregulation of LINE-1 results in the accumulation of reverse transcribed LINE-1 DNA in the cytosol which induces IFN-I in a neuroinflammatory disease, Aicardi-Goutières Syndrome. LINE-1 transcripts and proteins have also been shown to correlate with IFN-I expression in lupus nephritis kidney and Sjogren's salivary gland tissues. Our study characterized LINE-1 expression in patient samples with Systemic Lupus Erythematosus (SLE), Cutaneous Lupus Erythematosus (CLE) or Sjogren's Syndrome (SS) and evaluated the ability of the HIV-1 NRTI emtricitabine (FTC) to inhibit LINE-1 reverse transcriptase (RT) activity and reduce IFN-I production. **Results** LINE-1 transcripts were elevated in SLE patient whole blood samples compared with healthy controls in a published dataset (GSE72509). Total LINE-1 mRNA was also upregulated in CLE and SS patient whole blood samples at Gilead study enrollment (NCT03134222 and NCT03100942), compared to healthy controls. In vitro, FTC effectively inhibited LINE-1 RT activity in both the biochemical and the retrotransposition cellular assays. FTC also effectively suppressed the cGAS-STING pathway-dependent type I IFN production in demethylated human PBMCs. **Conclusion** Further studies will be required to determine the potential benefit of FTC, an HIV antiviral therapy, in lupus.

TH408. Cell Squeezing RBCs to Create Therapy for Inducing Antigen-Specific Tolerance

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Most autoimmune disease therapeutics are focused on broad immunosuppression and can have adverse effects and increase the risk of infection and cancer. Therefore, antigen-specific therapies that suppress autoreactive cells without altering the immune system is needed. The CELL SQUEEZE® technology utilizes microfluidic cell squeezing to engineer erythrocytes by encapsulating antigens and generate tolerizing antigen carriers (TACs). Cell squeezing makes the TACs resemble senescent RBCs enabling them to be cleared by the physiological mechanism of RBC clearance known as eryptosis. Here we show that TACs are rapidly cleared from circulation by phagocytic cells in the spleen and liver where the antigen is processed and presented to antigen-specific T cells in a tolerogenic fashion. Administration of TACs encapsulating the model antigens Ovalbumin (OVA) or Hen Egg Lyzozyme

(HEL) in OVA or HEL immunization models in various mice strains led to suppression of antigen-specific cytokine production by multiple tolerogenic mechanisms. Furthermore, the spleen was dispensable for TAC induced tolerance. TAC administration in an accelerated model of T1D delayed the onset of disease incidence. Our results suggest that TACs are a versatile platform for induction of antigen-specific tolerance.

TH409. Dual antiplatelet and steroid therapy in the management of Kawasaki's disease with G6PD deficiency

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Kawasaki's disease (KD) is an uncommon self-limited, vasculitis of unknown etiology that presents primarily in infants and young children with serious complications such as coronary artery aneurysms (CAA). The current recommended first-line therapy for treating acute KD and prevention of CAA is a combination of a single dose of intravenous immunoglobulin (IVIG) and moderate to high-dose aspirin. However, the use of high-dose aspirin to treat KD in children with concurrent Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency has not been well studied. G6PD deficiency is an x-linked recessive disorder that results in hemolysis caused by various triggers including aspirin, the mainstay of KD treatment. Alternative treatment regimens such as corticosteroids and other antiplatelet agents have been controversial, but clinical trials have demonstrated that the combination of methylprednisolone, IVIG, and aspirin might be beneficial in preventing CAA in patients resistant to initial IVIG. We present a 2-year-old Black male with concurrent KD and G6PD deficiency ultimately managed with dual antiplatelet therapy consisting of low-dose aspirin and clopidogrel, IVIG, and prednisolone after presenting with coronary artery dilation on outpatient follow-up. The patient had been initially managed with IVIG and prednisolone alone due to concerns for aspirin-induced hemolysis secondary to G6PD deficiency. Given the patient's positive clinical response and eventual resolution of CAA with the addition of dual antiplatelet therapy, we suggest further investigation into this alternative therapy for acute KD management, especially in patients with relative contraindications to high dose aspirin.

TH419. Interferon-α Stimulation of B cells in B6.Nba2 Lupus-like Disease Contributes to Splenomegaly, Auto-antibody Production, and Increased B cell Expression of Bcl-2

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Systemic Lupus Erythematous (SLE) is an autoimmune disease of unknown etiology affecting 5 million people worldwide. It is known that 50-70% of lupus patients present with increased interferon (IFN) stimulated genes, and that IFN-α receptor (IFNAR) gene deficiency can prevent lupus-like disease in multiple murine models. Multiple immune cells express IFNAR, but the effect of IFN-α stimulation and the disease manifestations caused by each cell type is unknown.

We used B cell specific IFNAR-deficient mice (BΔIFNAR) on the B6.Nba2 spontaneous lupus-like disease background to study how IFNαβ stimulation on B cells contributes to disease. Four month old BΔIFNAR mice displayed reduced levels of anti-dsDNA and anti-chromatin specific antibodies and significantly decreased splenomegaly. Furthermore, populations of germinal center B cells, memory B cells, plasma cell, and activated B cells were all significantly decreased in BΔIFNAR mice. Germinal center B cells from BΔIFNAR mice had significantly decreased expression of anti-apoptotic factor *Bcl2* and significantly increased expression of pro-apoptotic factor *Bim,* compared to B6.Nba2 diseased controls. In addition, *ex vivo* stimulation with recombinant IFN-α drastically increased intracellular BCL2 in sorted B cells from B6.Nba2 mice, but not BΔIFNAR mice. The increase in pro-apoptotic factors in B cells, in particular germinal center B cells, both *in vivo* and *ex vivo,* suggests that the reduction in the germinal center B cells, memory B cells, and plasma cell populations, and decreased autoantibodies seen in the BΔIFNAR mice is an effect of increased B cell apoptosis.

Cell and Gene Therapies

F27. CRISPR-based gene editing enables FOXP3 gene repair in HSPCs and IPEX patient T cells

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Forkhead box protein 3 (*FOXP3*) gene is a critical transcription factor for the function of thymic-derived regulatory T cell (Treg) and CD4+ effector T cell (Teff). Mutations in the *FOXP3* gene lead to the prototypical genetic autoimmune disease immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. IPEX is a severe and often fatal, pediatric disease with limited treatment options, including pharmacological immune suppression or allogenic hematopoietic stem cell (HSC) transplantation. As a monogenic immune disease, IPEX is an ideal candidate for a gene therapy approach whereby patient HSC or T cells are gene corrected and reinfused in the patient. However, engineering *FOXP3* should take into account spatiotemporal differential expression of the gene and a variety of diverse mutations scattered along the whole gene locus.

We developed a CRISPR/Cas9 approach combined with AAV delivery of a donor template to restore *FOXP3* expression at the endogenous locus, permitting regulated expression of wild-type FOXP3 irrespective of downstream mutations. Edited Tregs and Teff maintain their characteristic phenotypic markers and are functional *in vitro.* IPEX patient Tregs with different mutations are successfully edited

and achieve partial restoration of both FOXP3 expression and functional suppression. Gene edited cord blood hematopoietic stem and progenitor Cells (HSPCs) are capable of long-term engraftment in humanized mice and differentiate into multiple hematopoietic subsets (CD13, CD19, CD56, CD3) and partially reconstitute functional Tregs ($CD4^+CD25^+FOXP3^+$). These results demonstrate the feasibility of FOXP3 gene editing and will be instrumental for the development of therapeutic approaches for genetic autoimmune diseases such as IPEX syndrome.

F139. Factors that Affect the Ex Vivo Expansion of Alloantigen-Reactive Regulatory T Cells

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Regulatory T cells (Tregs) are being evaluated in different organ transplantation clinical trials to promote immune tolerance to transplanted grafts. Preclinical studies have shown improved efficacy of alloantigen-reactive Tregs (arTreg) over polyclonal Tregs in preventing graft rejection. Thus, our team has developed a GMP-compliant process of ex vivo expansion of arTregs where FACS-purified recipient-derived Tregs are first expanded with organ donor-derived stimulated B cells (sBc) for 11 days followed by 5 days of anti-CD3/CD28 bead-stimulated expansion. One major challenge identified during manufacturing of 18 clinical arTreg products was the highly variable rate of arTreg expansion ranging from 7- to 389-fold. arTreg expansion showed no correlation with any sBc attributes including sBc expansion, purity, or levels of CD80, CD86, or HLA-DR expression. To avoid confounding influence of Treg donor variability and properly investigate contribution of sBc quality to arTreg expansion, we stimulated Treg from same donors with a panel of different sBcs that represent a spectrum of sBc features. arTreg fold expansion ranged from 29 to 1620 with different sBc stimulations. arTreg expansion by day 11 showed no correlation with number of class II HLA mis-matches or HLA-DR expression levels on the sBcs, but positively correlated with sBc MFI of CD80 (R^2=0.8637, p=0.0073) and CD86 (R^2=0.9495, p=0.0010). This result reveals the importance of co-stimulation through CD28 in ex vivo arTreg expansion and the potential of improving arTreg product yield by enhancing co-stimulatory signal when sBc expression of CD80 and CD86 is not optimal.

F168. Expansion of Autologous Tumor Infiltrating γδ T cells in Peritoneal Surface Malignancies

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Background: Adoptive cell therapy employing αβ T cell receptor (TCR) expressing cells, recognizing major histocompatibility complex (MHC) restricted peptide antigen, is limited by neoantigen directed immune escape and tumor cells with mutational downregulation of MHC molecules. γδ T cells are primarily tissue resident lymphocytes that recognize metabolic intermediates and stress ligands by their

non-MHC restricted γδ TCR and natural killer receptors. We immunophenotyped tumor infiltrating γδ T cells and evaluated expansion strategies for potential use in cancer immunotherapy.

Methods & Results: Resected tumor fragments from patients with colon carcinomatosis (n = 5) or pseudomyxoma peritonei (n = 10) were assessed by multiparametric flow cytometry to assess viable tumor infiltrating lymphocyte (TIL) populations. Despite sparse overall infiltration of $CD3^+$ γδ TCR^+ cells (2-6%), interior rather than exterior tumor fragments contained more significant populations of γδ T cells. γδ T cells were negatively selected from proliferating TIL cultures and rapidly expanded with allogenic irradiated PBMC, OKT-3, and combinations of IL-2, IL-4, and IL-15, previously shown to enhance γδ T cell proliferation and effector function. Though γδ TIL grew slower than αβ TIL, a combination of IL-2 and IL-4 enhanced γδ TIL growth (450-fold expansion). Expanded γδ TIL were predominately $Vδ1^+$ $CD8^+$, and when compared to αβ TIL, maintained an effector memory phenotype ($CD62L^-$, $CD45RO^+$) and did not exhibit as extensive PD-1 expression.

Conclusion: Tumor infiltrating γδ T cells can be expanded from peritoneal surface malignancies, warranting study of their clonal diversity, metabolomics, tumor reactivity, and interactions with other immune populations.

F176. Subset characterization and functional testing of human regulatory T cells for adoptive immunotherapy

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Thymus-derived regulatory T cells (tTreg) play an important role in suppressing unwanted immune responses *in vivo*. Immunotherapies with human tTreg proved promising for the long-term prevention of autoimmunity and transplant rejection. First clinical adoptive transfer of *in vitro* expanded polyclonal autologous tTreg demonstrated safety and efficacy following renal transplantation.However, knowledge about the mode of tTreg differentiation would allow for a better prediction of tTreg fate following their application in adoptive tTreg transfer.

Applying conventional memory T cell-defining markers, we could demarcate naïve-, central memory- and effector memory-like tT_{REG} compartments as functionally distinct subsets presenting with distinct characteristics in terms of phenotype, lineage stability, functional capacities and epigenomic profile suggesting that tTreg underlie a pattern of linear differentiation. Intriguingly, we could identify a so far undescribed tTreg population within the naïve tTreg compartment demonstrating memory signature profiles.

Assessing the functionality of *in vitro* expanded tTreg is a vital quality release criterion prior to infusion for adoptive immunotherapy. While developing a robust and clinically feasible tTreg test system, we critically challenged a previously published and commercially available quick functional tTreg product release assay. Thereby, we showed suppression of early effector T cell activation markers and their

pro-inflammatory cytokine production to be inappropriate measures to determine tTreg functionality. Our data suggest that tTreg do not demonstrate assessable suppressive properties on early effector T cell activation, consequently there is ongoing need for optimization and development of novel, robust and feasible methods to evaluate tTreg function suitable for a GMP-compliant tTreg product release assay.

F178. Generation of powerful human tolerogenic dendritic cells by lentiviral vector-mediated human IL-10 gene transfer

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The prominent role of dendritic cells (DC) in promoting tolerance and the development of methods to generate clinical grade products allowed the clinical application of tolerogenic DC (tolDC)-based therapies for controlling unwanted immune responses. IL-10-secreting DC have been identified as the best-suited cells for tolDC-based therapies. We established an efficient method to generate tolerogenic human DC, producing supra-physiological levels of IL-10, by genetically engineering monocyte-derived DC with a bidirectional Lentiviral Vector (bdLV) encoding for IL-10 and amarker gene of selection (DC^{IL-10}). DC^{IL-10} are mature tolerogenic DC as shown by high expression of CD83, CD86, HLA-DR, ILT4 and HLA-G, that promote suppressive T regulatory type 1 (Tr1) cells. The tolerogenic profile and activity of DC^{IL-10} is maintained upon activation with different TLR stimuli or a mix of pro-inflammatory cytokines. In a humanized mouse model, adoptive transfer of human DC^{IL-10} dampen allogeneic T cell recall response, while murine DC^{IL-10} delays acute graft-versus-host disease in a C57BL/6 to BALB/c transplant mouse model. Our report outlines an efficient method to transduce human myeloid cells with large-size LV and shows that stable over-expression of IL-10 generates an effective cell product for future clinical applications in the contest of allogeneic transplantation.

F179. Dendritic Cell-based Antigen-Specific Immunotherapy to modulate T cell responses

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Tolerogenic Dendritic Cell (tolDC)-based approaches represent an innovative approach to modulate antigen(Ag)-specific T cell responses and induce tolerance in autoimmune diseases. To promote long-term Ag-specific tolerance tol DC must damp pathogenic T cell responses and promote/expand antigen-specific T regulatory cells.

We developed a method to genetically engineered DC with lentiviral vectors (LV) co-encoding for immunodominant peptide and the regulatory cytokine IL-10. To allow Ag-presentation and modulation of $CD4^+/CD8^+$ T cells, the peptide is fused to invariant chain (Ii).

Ag-specific tolDC, generated by transducing bone marrow cells with LV encoding for the model antigen Ovalbumin (OVA) alone (DC^{OVA}) or in combination with IL-10 ($DC^{IL\text{-}10/OVA}$), were functionally characterized. *In vitro* proliferation of OTI and OTII cells was reduced when stimulated with $DC^{IL\text{-}10/OVA}$ as compared to DC^{OVA}. To assess the ability of $DC^{IL\text{-}10/OVA}$ to modulate Ag-specific T cell responses *in vivo*, chimeric mice were generated by transplanting C57/Bl6 BM cells in combination with OTII or OTI BM cells and received repetitive DC injections. OTII and OTI cells expanded in mice receiving OVA-encoding DC, administration of $DC^{IL\text{-}10/OVA}$ induced expansion of OVA-specific Tr1 cells and of OVA-specific CD8+ exhausted T cells *in vivo*. Administration of DC engineered with LV encoding for Hybrid Insulin Peptides (HIP) or $InsB_{4\text{-}29}$ mimitope (containing the diabetogenic peptide $InsB9\text{-}23_{R22E}$) and IL-10 modulate the onset of autoimmune diabetes in pre-clinical models.

Our data provide a new method to generate IL-10-producing DC able to modulate pathogenic $CD8^+$ T cell responses and to induce Ag-specific Tr1 cells suitable for cell-based approach to restore tolerance.

F275. Regulatory Type 1 T Cell (T-allo10) Infusion in Mismatched Related or Unrelated Hematopoietic Stem Cell Transplantation (HSCT) for Hematologic Malignancies

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Novel strategies to reduce GvHD and improve long-term tolerance between mismatched donor-host pairs include regulatory T-cell therapy. T-allo10 is a cell product consisting of suppressive anergic cells enriched in IL-10 producing regulatory type 1 T-cells (Tr1). The main advantage of adoptive immunotherapy with Tr1 compared to other regulatory T-cells is the host alloantigen specificity that is established in the donor Tr1 during *in vitro* culture in the presence of IL-10 and host tolerogenic dendritic cells. Here we report the preliminary results of our phase I trial (IND 17292) on the use of escalating doses of T-allo10 cells for patients aged 3-45 years affected by hematological malignancies.

We completed the first cohort of the phase I portion of the study. The donors were class I or II single HLA-mismatched. The GvHD prophylaxis consisted of Sirolimus and Mycophenolate. Patients received $1x10^6$ T-allo10 cells/Kg on Day-1. No adverse events were observed after the T-allo10 cell infusion. All 3 patients met the safety criteria and are alive and disease-free at 2 years, 19 months and 5 months post-HSCT, respectively. Tr1, phenotypically defined as $CD4^+CD45RA^-CD49b^+LAG3^+$ were detectable in the peripheral blood shortly after the infusion at 21% and 26% in patient 2 and 3, respectively and IL-10 was detectable in the serum of the treated patients. TCR sequencing of T-allo10 cells prior and at multiple time points after infusion showed a restricted clonotype diversity within the Tr1 cells and persistence up to one year of certain clonotypes, demonstrating Tr1 cell recirculation and survival *in vivo*.

F294. CART19-BE-01 (NCT03144583): Experience with 47 Leukemia and Lymphoma Patients Treated in an Academic Clinical Trial Using a Closed Semi-Automatic Biorreactor Towards Hospital Exemption in Spain.

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In our CART19 academic clinical trial, **CART19-BE-01 (NCT03144583)**, we used for production a semi-automated process (CliniMACS Prodigy) to treat 47 patients with ALL, NHL and CLL. *Ex vivo* cell expansion lasted an average of 8.5 d and had a mean transduction rate of 30.6%±13.44. All products obtained presented cytotoxic activity against CD19+ cells, secreting cytokines after target recognition. Although expansion kinetics was slower in patients, potency was comparable. CAR T-cell phenotype was highly variable among patients and largely determined by the initial product. TCM and TEM were the predominant phenotypes. An in-depth analysis revealed that e*x vivo* expansion leads to reduced numbers of TN, TSCM and TEFF, while TCM increase, both due to cell expansion and CAR-expression. Overall, our results show for the first time that clinical-grade CART production for heavily pre-treated patients using CliniMACS Prodigy system is feasible and with the required quality. Reduced *ex vivo* expansion may yield CAR T-cell products with increased persistence *in vivo*. Expansion time: 8.5 days (7-10); mean number of total obtained CARTs: $870x10^6$ (120-1928); with a target cell dose of $1x10^6$/kg CARTs for ALL and $5x10^6$/kg CARTs for NHL and CLL, consecutive fractionated doses (10%+30%+60%) clearly reduced adverse effects. As a summary, in 37 ALL patients (25 adults), 5 patients do not developed clinical responses, while Complete Responses at day 100 were 71% and

actuarial probabilities at 1y were 8% (TRM), 45% (PFS) and 84% (OS). This proposal of treatment is under evaluation by Spanish drug agency (AEMPS) for hospital exemption.

TH1. Using Precision Genome Editing to Interrogate Regulators of Human Treg Stability

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Regulatory T cell (Treg) therapy is a promising curative approach for a variety of immunopathologies, including transplant rejection and autoimmunity. As Tregs move into clinical testing, an outstanding concern is their potential to convert into pathological effector T cells in vivo. It is imperative to understand the factors governing Treg stability. We and others have shown that phosphatases such as PHLPP1 and PTEN dampen PI3K-AKT activity to maintain Treg stability and suppressive function in mice, but how these regulators operate in humans remains unclear. To this end, using human Tregs, we optimized a method to efficiently deliver CRISPR/Cas9 ribonucleoprotein by electroporation and a homology-directed repair (HDR) template by adeno-associated virus to knock-out a gene of interest and, in parallel, knock-in a reporter gene to achieve a uniform population of gene-KO Tregs after flow sorting. Using *FOXP3* as a proof-of-principle target, we achieved 40-50% HDR (GFP^+) in Tregs with a concurrent loss of protein expression. As expected, $FOXP3^{KO}$ Tregs lost their suppressive capacity. Using this platform, we generated $PHLPP1^{KO}$ Tregs; preliminary data suggests that PHLPP1 safeguards Tregs from spontaneous proliferation and inflammatory cytokine expression in vitro. Future work will examine the metabolic consequences of *PHLPP1* genetic ablation and compare this phenotype to other potential regulators of PI3K-AKT activity in human Tregs, including PTEN. Identifying the mechanisms by which human Tregs maintain their lineage stability will help uncover therapeutic targets to improve the efficacy of Treg cell therapies.

TH4. IL-10-related Regulatory Cells in Celiac Disease: Role in Controlling Gliadin-Specific T Cell Responses

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Celiac Disease (CD) is a T-cell mediated disorder caused by an altered immune response to gluten in genetically predisposed individuals. Despite positive serology and genetics, 10% of CD subjects display a healthy intestinal mucosa (potential CD, PCD). Since the mechanisms underlying the divergent evolution of disease in PCD *vs* active forms and in patients responsive or refractory to the gluten-free diet (GFD) are not yet clarified, we are currently investigating the presence and function of IL-10-related regulatory cells, both T cells and tolerogenic (Tol) DC, in the peripheral blood and gut mucosa of CD patients at different stage of the disease (i.e., PCD, atrophic mucosa, GFD). Preliminary data suggest increased frequency of DC-10 (i.e. tolDC subset constitutively secreting IL-10) and FOXP3$^+$ T cells infiltrating the gut mucosa of PCD and CD patients with atrophic mucosa, respectively. Furthermore, with the aim of generating tolDC suitable for cell-based therapy of CD, we transduced monocytes derived from the peripheral blood of CD patients with lentiviral-vectors (LV) enforcing HLA-class-II restricted presentation of gliadin-derived epitopes and differentiated them into DC in the absence (DC^{Ag}) or presence of transgenic IL-10 ($DC^{IL\text{-}10/Ag}$). $DC^{IL\text{-}10/Ag}$ expressed tolDC associated markers, induced a hypo-proliferative phenotype and promoted the expansion of Ag-specific CD49b$^+$LAG3$^+$ Tr1-like cells. Our study will provide new insights into the mechanisms underlying CD progression and response to GFD, will identify biomarkers of disease progression to better target patients for GFD and will open new perspectives for the therapy of CD.

TH28. Human engineered Treg-like cells suppress FOXP3 deficient T-cells and preserve adaptive immune responses in humanized mice

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FOXP3 is an essential transcription factor for regulatory T cell (Treg) function and a key regulator of immune tolerance. Genetic or acquired defects in Treg play a key role in many immune mediated diseases including Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome. The use of Treg has been exploited in the clinic, in preventing Graft-versus-host disease (GvHD) or controlling autoimmunity. Treg cell therapy, either with freshly isolated or *in vitro* expanded Tregs have been proven to be feasible and safe. However, current challenges remain in isolating/generating sufficient number of pure Treg cells and in obtaining a stable cell product. To overcome these hurdles, we have previously demonstrated that CD4+ T cells from healthy donors and IPEX patients can be converted into functional Treg-like cells by lentiviral transfer of *FOXP3* ($CD4^{LVFOXP3}$). Here, we demonstrate that this conversion is associated with upregulation of 7 Treg genes including *FOXP3*, and the preservation of a polyclonal TCR repertoire. Both allogeneic and autologous $CD4^{LVFOXP3}$ protect from xeno-GvHD at first and second challenge with effector T cells. $CD4^{LVFOXP3}$ prevent hyper-proliferation of CD4$^+$memory T-cells in a novel IPEX-like humanized-mouse model. However, $CD4^{LVFOXP3}$ do not impede immune responses to various pathogens or tumor clearance. These data

strongly support the clinical use of $CD4^{LVFOXP3}$ to treat immune-mediated diseases caused by insufficient or dysfunctional $FOXP3^{+}$Tregs.Therefore, IPEX is an ideal disease candidate for a Proof-of-concept trial of $CD4^{LV\text{-}FOXP3}$ cell therapy and for setting the stage to a broader application of $CD4^{LVFOXP3}$ in immune-mediated disorders of different origin.

TH36. Immunosuppressant-resistant regulatory T cell products for improved functionality in transplant recipients

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To date, the majority of solid organ transplant (SOT) recipients depends on lifelong immunosuppressive medication to prevent rejection of the allogenic organ. Recent adoptive cell therapy approaches using regulatory T cells (T_{reg}) in humans imply that classical immunosuppressive medication, which has many adverse effects, may be minimized, but cannot yet be completely abolished in clinical trials. Standard immunosuppressants also harm adoptively transferred T_{reg} as their target structures are not restricted to pathogenic allogen-reactive T cells and similarly important for T_{reg}. In this project, we aim to improve performance of T_{reg} products in transplant recipients by induction of resistance to immunosuppressants. Therefore, we performed CRISPR-Cas9-mediated gene-editing to knock-out (k.o.) an adaptor protein required for function of a particular immunosuppressive drug. We developed a GMP-compliant sorting strategy to obtain pure T_{reg} from which we successfully generated k.o. T_{reg} products by transfer of nucleoprotein complexes of Cas9 and a site-specific single guide RNA resulting in vector-free k.o. of the cell-intrinsic target protein. Assessment of phenotype, characteristics and functional attributes of k.o. T_{reg} products revealed improved expansion and elevated expression of functional T_{reg} markers on k.o. T_{reg} when cultured in the presence of the target immunosuppressant implying possibly enhanced functionality in transplant recipients. The preclinical proof-of-concept shall be achieved in a GvHD mouse model. Currently, we investigate potential off-target effects, compare the transcriptome of k.o. and untouched T_{reg} products and prepare GMP-compliant translation of k.o T_{reg} products to a first-in-human application.

TH40. Engineered Type-1 Regulatory T Cells as Cellular Therapy for Treatment of Immune Mediated Diseases.

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Type 1 regulatory cells (Tr1) are a promising cellular product for suppression of effector T cells in immune mediated diseases, including graft-versus-host-disease (GvHD) in allogeneic hematopoietic stem cell transplantation (allo-HSCT) (Roncarolo et al. *Immunity* 2018). We have developed an *in vitro* protocol to produce Tr1 cells by lentiviral transduction of the human *IL10* with a constitutive promoter into human $CD4^+$ T cells (Locafaro et al. *Molecular Therapy* 2017). These engineered Tr1 cells (LV10) acquire a characteristic Tr1 cytokine profile (IL-10 and IFN-g high, IL-4 low and expression of intracellular perforin and granzyme B). *In vitro*, LV-10 cells suppress proliferation of responder $CD4^+$ T cells upon activation by allogeneic dendritic cells. LV-10 cells also degranulate in response to and kill myeloid cells, including myeloid blasts from patients with acute myeloid leukemia, through a granzyme B- and perforin-dependent mechanism. Interestingly, the ability to degranulate and kill myeloid cells is not present when LV-10 are activated and expanded with CD3 and CD28 coated beads, suggesting that signals beyond TCR, CD28, and IL-10 receptor pathway activation are necessary to reprogram LV-10 cells into cytotoxic cells. *In vivo*, LV-10 cells injected into NSG mice do not induce xeno-GvHD, in contrast to control $CD4^+$ T cells. LV-10 cells also suppress CD4-induced xeno-GvHD and prevent expansion of myeloid leukemic cells. Experiments are ongoing to compare the potency and *in vivo* survival of allogeneic vs autologous LV-10 cells. These findings demonstrate the promise of using LV-10 to treat immune mediated diseases, including GvHD in patients receiving allo-HSCT.

TH59. Novel Bioluminescent Bioassays for the Discovery and Development of T Cell Redirecting Cancer Therapies

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Redirected T cell therapies include anti-CD3 bispecific molecules (such as BiTE), CAR-T and TCR-T therapies). BiTEs redirect the cytotoxic activity of T cells by simultaneously engaging CD3 on T cells and tumor antigens on target cells. BiTE potency studies have relied on primary cells, which measure target cell killing through redirected T cell cytotoxicity (RTCC) or cytokine release. However, these primary cell-based assays suffer from high donor variability, as well as complex assay protocols. We have developed a new RTCC assay and cytokine immunoassays that are simple, sensitive and quantitatively measure the potency of BiTEs and similar biologics. Specifically, preactivated cytotoxic T cells and target cells stably expressing a HaloTag-HiBiT fusion protein are co-incubated with a BiTE, which results in lysis of the target cells and release of the Halotag-HiBiT protein. These HiBiT proteins then bind to extracellular LgBiT in the detection reagent forming functional NanoLuc and a bioluminescent signal. The assay is homogenous, highly sensitive, and has a robust assay window. To facilitate the screening and characterization of new transgenic TCRs, we used CRISPR/Cas9 to develop two TCRαβ-null reporter T cell lines (CD4+ or CD8+). Reintroduction of peptide-specific TCR α and β chains results in peptide-dependent TCR activation and luciferase reporter expression. The select expression of CD4 or CD8 in the TCRαβ-null reporter T cell lines enables the development of transgenic TCRs for both MHCI- and MHCII-restricted tumor antigen targets. Together, these bioluminescent bioassays represent a new set of tools for the discovery and development of T cell-based immunotherapies.

TH62. Type 1 Regulatory T Cell-Based Therapy for Pediatric AML

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Pediatric acute myeloid leukemia (pAML) accounts for nearly half of the deaths from leukemia in children. Relapsed or treatment-refractory pAML is treated with an allogenic hematopoietic stem cell transplant (HSCT); however, balancing the graft's ability to clear residual leukemia while protecting host tissues from graft versus host disease is a challenging task. We developed a type 1 regulatory T cell-based therapy, called LV10, by enforcing constitutive expression of IL-10 in CD4+ T cells. We found that LV10 kill myeloid cells, including primary AML. In this study, we tested the ability of LV10 to lyse 23 primary pAML *in vitro* and observed distinct killing sensitivities. The pAML sensitivity to LV10 killing did not correlate with pAML genomic alterations or classic risk stratifications, but our transcriptome analyses by RNA sequencing suggest that resistance to lysis is intrinsic to the pAML's molecular profile either by upregulating genes that inhibit LV10 function, or downregulating genes required for LV10-mediated killing or recognition. We explored potential cell surface molecules that may inhibit LV10 function and after overexpressing specific surface molecules on LV10-sensitive myeloid cell lines, we observed a reduction in LV10 cytotoxicity. We found that the molecular signatures of the sensitive and resistant pAML were reflected in the pAML dataset produced by the Office of Cancer Genomics Therapeutically Applicable Research to Generate Effective Therapy, suggesting that sensitive AML have a more differentiated myeloid phenotype. These results show that pAML can be targeted by LV10 cells, supporting the use of LV10 based therapy for pAML undergoing HSCT.

TH69. Stabilizing the phenotype of human regulatory T cells in the presence of inflammatory triggers through targeted deletion of switchpoint receptors

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Adoptive transfer of regulatory T cells (Treg) is a promising strategy to treat autoimmune diseases. Inflammatory environments can induce the polarization of Treg cells into Th17 and Th2-like cells via signaling through Treg "switchpoints". This plasticity of the regulatory T cell phenotype is a major concern whenever antigen specific Treg products are employed clinically. Multiple humoral and cellular signaling pathways have been implicated in the Treg de-polarization. Here, we evaluate the effect of individual deletion of four different receptors implicated in Treg plasticity on their proliferation and phenotype. Deletion of Treg switchpoints is performed using electroporation of CRISPR-Cas ribonucleoproteins. Subsequently, the stability of switchpoint-modified Treg cells is assessed in the

presence of known inflammatory triggers to select the two most promising switchpoint candidates for further preclinical evaluation.

TH81. Optimal tolDCs and their application routes for prevention of type 1 diabetes (T1D) in the NOD and NOD/SCID mouse models

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Tolerogenic dendritic cells (tolDCs) are explored as a promising cell therapy in treatment of autoimmune diseases including type 1 diabetes (T1D). T1D is a T-cell-mediated, organ-specific disease characterized by destruction of insulin-producing beta cells. Several tolDCs protocols have been tested for prevention or cure of T1D in animal models. We tested effectiveness of antigen-loaded (GAD65) and unloaded tolDCs to prevent diabetes in the NOD-SCID model of adoptive cotransfer and in spontaneously diabetic NOD mice. Next we have compared various application routes of unloaded tolDCs to migrate to the critically important draining pancreatic lymph nodes and to prevent T1D. TolDCs were stained with PKH26 and migration of 4x106 labeled cells was followed by flow cytometry after i.p., i.v., s.c. left and s.c. right side of the body applications on day 1, 3, 5, 7, and 9 in spleens, pancreatic (PLN), mesenteric (MLN), inguinal (ILN), and axillar (ALN) lymph nodes. Live PKH26+CD11c+CD3- cells were preferentially detected in spleens and PLNs after i.p. and i.v. administrations, whereas s.c. injections led to the accumulation of tolDCs in ILNs and ALNs on the corresponding application side. These data were confirmed by visualization of CFSE-labeled tolDCs by Light-sheet microscopy. The effect of application route on the diabetes prevention was tested in both the NOD-SCID and NOD models being most effective for the i.p. and i.v. administrations. Our data document that both antigen loading and application routes affect effectiveness of tolDCs protocols that should be optimized in animal models before their translation to clinical testing.

TH84. Chimeric antigen receptor signaling confers antitumor activity to human regulatory T cells

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Regulatory T cell (Treg) therapy offers the opportunity to treat transplant rejection and autoimmunity without the toxicity of immunosuppressive regimens. Conferring antigen specificity to Tregs using a chimeric antigen receptor (CAR) dramatically expands what targets can be pursued. However, the consequences of CAR-mediated signaling for human Treg biology remain poorly understood. To address this question, we generated anti-CD19 CAR Tregs with a tandem CD28-TCRz intracellular

domain. Upon *in vitro* co-incubation with CD19-expressing cells, CAR Tregs upregulated activation markers, proliferated, secreted IL-10, and suppressed T cell proliferation, while maintaining high FOXP3 expression and a demethylated TSDR. In humanized mice harboring CD19-expressing tumor cells, CAR Tregs suppressed CAR T cell proliferation. Strikingly, CAR Tregs also suppressed tumor growth, even in the absence of CAR T cells, for three tumor cell types (B-cell leukemia, myeloid leukemia, and epithelial carcinoma) and two routes of delivery (intravenous and subcutaneous). Real-time monitoring *in vitro* confirmed that CAR Tregs suppress tumor cell growth. Surprisingly, CAR Tregs controlled the growth of CD19-negative tumor cells *in vivo* when co-administered with CD19-expressing cells, indicating bystander effects. Single-cell cytokine analysis revealed that CAR-mediated Treg activation leads to higher production of IFN-g, TNF-a, perforin, and granzyme B than anti-CD3/28 stimulation. CRISPR/Cas9-mediated ablation of these molecules has been performed to test their role in CAR Treg antitumor activity *in vitro* and *in vivo*. Experiments evaluating *in vivo* CAR Treg stability and impact on normal tissues are underway. Overall, these observations indicate that CAR Treg cytotoxicity is an important concern for safe clinical translation.

TH87. Differentiation, Expansion and Stability of In vitro Allo-specific Tr1 Cells with potential use in cellular therapy.

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T regulatory type 1 (Tr1) cells are considered one of the best candidates for cellular therapy. Tr1 cells are Foxp3- T cells that can be identified by the co-expression of CD49b and LAG-3 and high production of IL-10. A recent protocol based on the co-culture of naïve T cells with allogeneic DC-10 which allows efficient Tr1 differentiation *in vitro*, has been approved for clinical trials. However, $CD49^{+}LAG3^{+}$ Tr1 cells are highly heterogeneous and a suppressive subpopulation has been recently characterized, which co-expresses several co-inhibitory receptors including PD-1, CTLA-4, CD39, TIM-3 and TIGIT, and produces high levels of IL-10.

Here we established an optimized protocol that allows *in vitro* differentiation and long-term polyclonal expansion of allospecific Tr1, showing high suppressive function and stability under pro-inflammatory conditions. Co-culture of allogeneic naïve T with DC_{10} human cells in the presence of IL-10 resulted in >40% of CD49b+ LAG-3+ Tr1 cells. Polyclonal stimulation of sorted allospecific Tr1 cells achieved a 1000x expansion and phenotypic analysis showed that 80% of the expanded cells showed expression of co-inhibitory molecules PD-1, CTLA-4, TIM-3, TIGIT, CD39. Interestingly, 90% of Tr1 expanded cells expressed significant levels of IL-10. Moreover, when we evaluated the stability of this population in the presence of pro-inflammatory cytokines (IL-1β, IL-6, and IFN-γ) we found that Tr1 cells maintained the expression of all co-inhibitory molecules and IL-10 production. Finally, we found significant suppression of allospecific T cell proliferation, indicating that these Tr1 cells may be considered as potential candidates for their use as immune therapy.

TH90. A new interpretable machine learning method for automated cell population discovery identifies correlates of clinical outcome in cancer immunotherapy

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High-dimensional single-cell cytometry is routinely used to characterize patient responses to cancer immunotherapy and other treatments. This has produced a wealth of datasets ripe for exploration but whose biological and technical heterogeneity make them difficult to analyze with. We introduce a new interpretable machine learning method for single-cell mass and flow cytometry studies, FAUST, that robustly performs unbiased cell population discovery and annotation and enables data integration across studies and platforms. FAUST returns biologically interpretable cell phenotypes that can be compared across studies, making it well-suited for the analysis and integration of complex datasets. We use FAUST to perform candidate biomarker discovery and validation by applying it to flow and mass cytometry datasets from melanoma anti-PD-1 trials in order to discover and validate new CD4+ and CD8+ effector-memory T cell correlates of outcome co-expressing PD-1, HLA-DR, and CD28. Comparisons with existing state-of-the-art computational discovery approaches as well as prior manual analyses did not detect any statistically significant T cell correlates associated with anti-PD-1 treatment in either data set. We further validate our approach by using FAUST to replicate the discovery of previously reported myeloid correlates in a third published melanoma trial, and then validate these by identifying them de novo in two additional independent trials. FAUST enables cross-study data integration in the presence of heterogeneous data and diverse immunophenotyping staining panels, enabling hypothesis-driven inference about cell sub-population abundance through a multivariate modeling framework we call Phenotypic and Functional Differential Abundance (PFDA). We showcase this approach on data from multiple trials.

TH92. Low-dose radiation improves CD19-targeting CAR T cells expansion and leukemia control in mice

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Purpose/Objectives: Low-dose radiation therapy (LD-RT) has been shown to enable antigen-independent killing by CAR T cells as a consequence of death receptor (DR) upregulation in a model of pancreatic adenocarcinoma. These findings have major implications for the management of antigen escape as a mechanism of resistance to clinically approved CAR T cells. We therefore set to test the ability of LD-RT to improve the ability of CD19-specific CAR T cells to control leukemia following DR upregulation.

Materials/Methods: We harnessed GFP^{+}Nalm6 leukemia cells to determine the ability of LD(1Gy)-RT to cause DR upregulation *in vitro*and*in vivo*. We combined LD-RT with CAR T cells *in vivo*, in NGS mice bearing GFP^{+}Nalm6 cells and monitored CAR T cell expansion, leukemic burden and overall survival.

Results:*In vitro* and *in vivo*, LD-RT caused the upregulation of DRs, mainly FAS and TRAIL-R2, on Nalm6 cells, with TRAIL-R2 upregulation persisting *in vivo* for up to 11 days after irradiation. LD-RT delivered to leukemia-bearing mice a few hours before CAR T cell infusion significantly extended overall survival (median 110 days) as compared to CAR T cell infusion alone (median 41 days), correlating with superior CAR T cell expansion (p=0.005). The CAR T expansion was also documented in leukemia-naïve mice. Conversely, LD-RT administered at disease relapse after CAR T cell infusion was not able to extend survival as compared to CAR T cell infusion alone.

Conclusions:LD-RT delivered before CAR T cells supports CAR T cell expansion and considerably improves therapeutic activity.

TH93. Targeting CLL-1 using chimeric antigen receptor T cells (CART) in acute myeloid leukemia

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Acute myeloid leukemia (AML) is a disease with high incidence of relapse that is originated and maintained from leukemia stem cells (LSCs). Hematopoietic stem cells (HSCs) can be distinguished from LSCs by unique antigen expression such as CD123 and CLL-1. Thus, these are targets of interest to eliminate LSCs without harming HSCs. Here, we evaluated the potential of CLL-1 as a therapeutic target for LSCs using allogeneic chimeric antigen receptor (CAR) T cells (UCARTCLL1). UCARTCLL1 cells are genetically modified allogeneic T-cells expressing an anti-CLL-1 CAR and that lack expression of the T-cell receptor (TCRαβ^{neg}) and of the Class I Major Histocompatibility Complex (MHC-I). We first evaluated the expression of CLL-1 on blasts and phenotypically defined stem cells (CD34^{pos} CD38^{neg}) from 41 AML patients and 4 healthy donors using multi-parameter flow cytometry. Furthermore, we found that 35 out of 41 primary AML samples had phenotypically defined CLL1^{pos}CD34^{pos}CD38^{neg} stem cells. Next, we evaluated cytotoxic efficacy of UCARTCLL1 cells at different effector to target ratios in 7 primary AML samples with different levels of CLL-1 expression. We observed a significant cytotoxic effect specific to the CLL1^{pos} subsets. Notably, CLL-1^{pos} cells in the AML samples treated with 5:1 and 1:1 ratios were completely eliminated. Currently, we are evaluating the in vivo activity of UCARTCLL1 cells in AML-PDX mouse models to determine their efficacy at eliminating AML blasts, progenitors and LSCs.

TH161. Cell Therapy using CD8+Tregs in Human Transplantation

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$CD8^+CD45RC^{low}$T cells have been described by us and others as highly suppressive cells in rodent and human. Importantly, we highlighted their therapeutic potential in transplantation models of human skin graft allogeneic rejection and human PBMCs-induced GVHD in NSG mice (Bézie et al., Frontiers Immunol., 2018). More recently, we have shown that conferring specificity toward a HLA mismatch in transplantation by using the CAR technology significantly improved their therapeutic efficiency (Bézie et al., Blood Advances, 2019). To date, there has been no clinical trial using $CD8^+$Tregs. Thus, our goal was to develop a GMP-compatible protocol to launch a FIH clinical trial using $CD8^+$Treg cell therapy in transplantation as a part of the H2020 RESHAPE program.

We sat up a process to isolate and expand circulating $CD8^+$Tregs in GMP conditions. Based on cell expansion yield, Treg-associated marker expression and suppressive function, we selected a clinically applicable culture medium combined with serum, cytokines and chemical supplements. In addition, IL-2 and rapamycin doses and stimulation methods were determined and critical to preserve their function. In vivo, we observed that $CD8^+$Tregs persist for more than 80 days in NSG mice lacking human cytokines. Finally, we demonstrated in vitro and in vivo that allogeneic off the shelf $CD8^+$Tregs can be used in cell therapy.

At the dawn of the FIH clinical trial using $CD8^+$Treg cell therapy, we are already working on the next generation of $CD8^+$Tregs for transplanted patients with genetic modifications to secure function, stability, high persistence and specificity.

TH331. Islet-specific Engineered Regulatory T Cells Suppress Proliferation of Pathogenic Effector T Cells and Block Type 1 Diabetes

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In comparison to adoptive transfer of polyclonal regulatory T cells (T_{reg}), murine antigen (Ag)-specific T_{reg} exhibit superior efficacy in blocking or reversing Type 1 diabetes (T1D) in animal models. However, human T_{reg} specific for a particular antigen are rare and in vitro expansion poses a potential risk of loss of FOXP3 expression and function. To address these challenges, we have developed islet-Ag-specific engineered T_{reg} (edT_{reg}) with stable FOXP3 expression. Homology-directed repair (HDR)-editing was utilized to integrate a strong promoter, MND, within the *Foxp3* locus enforcing stable, high-level, FOXP3

expression resulting in a T_{reg}-like phenotype and functional activity. Polyclonal or islet-Ag-specific edT_{reg} were generated from $CD4^+$ T cells from NOD and NOD.BDC2.5$^+$ mice, respectively. Following adoptive transfer, islet-specific edT_{reg} homed to pancreas and persisted. Both islet-specific edT_{reg} and nT_{reg} blocked diabetes triggered by islet-specific T_{eff} in immunodeficient recipient mice while polyclonal edT_{reg} or nT_{reg} failed to do so. As proof-of-concept in human cells, HDR-editing was utilized to generate edT_{reg} expressing islet-specific TCRs obtained from CD4+ T cells derived from T1D subjects. Results from in vitro assays showed that islet-specific edT_{reg} suppressed proliferation and cytokine production by islet-specific T_{eff}; strikingly, edT_{reg} also suppress polyclonal islet-specific T cells derived from PBMC. edT_{reg} suppressed T_{eff} recognizing the identical peptide as well as bystander T_{eff} recognizing alternative antigens. Further, edT_{reg} TCRs with higher avidity had superior suppressive capacity compared with those with lower avidity. In summary, islet-specific edT_{reg} efficiently prevent T_{eff} activation in similar manner as nT_{reg} and comprise a promising therapeutic approach for T1D.

TH380. Impact of Receptor Signaling Intensity on the Suppressive Activity of Engineered Receptor Regulatory T Cells

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Adoptive transfer of polyclonal regulatory T cells (Treg) have demonstrated promising outcomes in autoimmune diseases, transplantation and graft versus host disease (GvHD). Polyclonal Tregs can be engineered into antigen specific Tregs by incorporation of recombinant receptors like chimeric antigen receptor (CAR) or T cell receptor fusion constructs (TRuCs), thus overcoming the limitations of polyclonal Treg therapy. Using hemophilia as a model, we sought to engineer antigen-specific Tregs to suppress neutralizing antibody (inhibitor) formation against the soluble therapeutic clotting protein, factor VIII (FVIII) in an MHC-independent fashion. Surprisingly, CAR Treg engagement with a high-affinity receptor resulted in heightened signaling and a robust effector phenotype *in vitro* that was distinct from the activation signature observed for endogenous thymic Tregs, and resulted in the loss of suppressive activity. Cellular therapy with these high affinity CAR Tregs in a murine model of severe hemophilia A exacerbated the immune response to therapeutic FVIII protein by generating high titer inhibitors. Targeted mutations in the CD3ζ immune receptor tyrosine-based activation motif (ITAMs), CD28 phospho-tyrosine residues or IL-10 overexpression were not sufficient to restore suppressive potential of FVIII CAR Tregs. In contrast, complexing TCR-based signaling with FVIII specific single chain variable fragment (scFv) recognition to generate TRuC Tregs was able to deliver controlled antigen-specific signaling via engagement of the complete TCR complex, resulting in functional suppression of the FVIII-directed antibody response. These results suggest that adoptive cellular therapies with antigen specific engineered Tregs for tolerance induction will require regulated activation thresholds to maintain optimal suppressive function.

COVID-19

F390. Potential Role of Exosome Extracellular Vesicles in Convalescent Plasma Therapy of COVID-19

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Introduction

In the pandemic rush to use Convalescent Plasma Therapy (CPT) for COVID-19 patients now with premature FDA approval despite insufficient non-scientific studies, a further problem concerns the content of what is transfused.

Methods

Compared to prior times, we now know that there is a lot more to the protective immune system than mere antibodies; so far the whole focus of CPT. Per COVID-19 the dominant cellular immune system of viral specific immune T cells activated by antigen presenting cells (APC) is represented by "convalescent exosomes" (Exos) able to transfer RNAs, they likely mediate some of the healing cell-mediated aspects.

Results

Past trials of CPT have provided choppy data, and some now show little or no benefit; including the Mayo Clinic led FDA trial upon which their positive decision was based. Further, there are many observations to doubt how important the Ab are, like the recovery from COVID-19 of patients unable to mount an Ab response.

Conclusions

It is an unfortunate oversight to not consider the acquired immune functions of the billions of bioactive Exos present in convalescent plasma likely participating in the immune responses to COVID-19 that CPT is aiming to alter.

F402. B cell subsets as severity-associated signatures in COVID-19 patients.

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Background. SARS-CoV-2 infection represents a global health problem that has affected millions of people. The fine host immune response and its association with the disease course have not yet been fully elucidated. Consequently, we analyze circulating B cell subsets and their possible relationship with COVID-19 features and severity.

Methods. By using a multiparametric flow cytometric approach, we determined B cell subsets frequencies from 52 COVID-19 patients, grouped them by hierarchical cluster analysis and correlated their values with clinical data.

Results. The frequency of $CD19^+$ B cells is increased in severe COVID-19 compared to mild cases. Specific subsets such as transitional B cell subsets increase in mild/moderate cases but decrease with severity of the disease. Memory B compartment decreased in severe and critical cases and antibody secreting cells are increased according to the severity of the disease as well. Other non-typical subsets such as double negative B cells also showed significant changes according to disease severity. Globally, these differences allow us to identify severity-associated patient clusters with specific altered subsets. Finally, respiratory parameters, biomarkers of inflammation and clinical scores exhibited correlations with some of these subpopulations.

Conclusion. The severity of COVID-19 is accompanied by changes in the B cell subpopulations, either immature or terminally differentiated. Furthermore, the existing relationship of B cell subset frequencies with clinical and laboratory parameters, suggest that these lymphocytes could serve as potential biomarkers and even active participants in the antiviral adaptive response mounted against SARS-CoV-2.

F405. Immunological Risk Profile in COVID-19, the Experience of a Single Academic Hospital in Spain

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One of the main difficulties in the management of patients with COVID-19 is the shortage of tools to anticipate hyperinflammation or to prioritize ICU beds. Acute phase reactants (APR), hematological parameters and IL-6 have been considered the most suitable. Prognostic power of demographic data and 32 laboratory parameters in relation to the final outcome (discharge home vs exitus) have been analyzed in 1758 consecutive patients >18 years (790F/968M, mean age 62.5 ± 16.9) admitted to the emergency dept of a single hospital during the first wave of pandemic. Patients with major concomitant diseases -but no comorbidities- were excluded. Age was the main demographic determinant of prognosis (mean age discharged 60.86 versus exitus 74.5). IL-6, CRP, Ferritin, D-dimer, Lymphopenia and Neutrophil/Lymphocyte Ratio were associated to outcome in univariate and multivariate analysis. In principal component analysis, the Dm1 and Dm2 explained the 20.8% the 10.8% of the prognosis. Neutrophil and lymphocyte parameters followed by APR were the main determinants. In the random forest model of classification, the parameters with higher coefficients were log IL-6, age, urea, LDH, neutrophils, Hb, monocytes, lymphocytes and CRP. Some parameters typically associated to hemophagocytic lymphohistiocytosis i.e. liver tests, triglycerides and low platelets were less strongly associated. These results indicate that laboratory parameters and age can be used to generate a predictive algorithm but more parameters, e.g. additional inflammatory cytokines, ideally closer to the pathophysiological determinants of severity, may be required to improve prediction power. Obviously, image technique and clinical monitoring remain the cornerstones for prognosis.

F421. The COVID-19 Immune Landscape Is Dynamically and Reversibly Correlated With Disease Severity

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Despite a rapidly growing body of literature on the COVID-19 pandemic, our understanding of the immune correlates of disease severity, course and outcome remains poor. Using mass cytometry, we assessed the immune landscape in longitudinal whole blood specimens from 59 patients presenting with acute COVID-19. Patients were classified based on maximal disease severity using a 7 point ordinal scale with 24 patients with severe disease, 28 patients with moderate disease and 7 patients with mild disease. Hospitalized patients negative for SARS-CoV-2 were used as controls. Notably, we found that the immune landscape in COVID-19 forms three dominant clusters, which correlate with disease severity. Longitudinal analysis identified a pattern of a productive innate and adaptive immune responses in individuals who have a moderate disease course, whereas those with severe disease have features suggestive of a protracted and dysregulated immune response. Further, we identified immune-alterations accompanying clinical improvement that were also seen in patients who received both IL-6 pathway blockade and convalescent plasma infusion, that were not present in those receiving convalescent plasma alone. Interestingly, we found features such as neutrophilia and lymphopenia were also present in severely ill hospitalized patients without COVID-19, but also identified immune features that were unique to severe COVID-19. Importantly, our findings indicate that selection of immune interventions should be based in part on disease presentation and early disease trajectory due to the profound differences in the immune response in those with mild to moderate disease and those with the most severe disease.

F436. Cytokine profile of COVID-19-related MIS-C compared with pre-pandemic Kawasaki disease and SARS-CoV-2 specific immune complexes detection in MIS-C.

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A multisystem inflammatory syndrome (MIS-C) with temporary association with SARS-CoV-2 pandemics has been recently described in children, it shares some common features with Kawasaki disease (KD). We hypothesized that: 1) the immune cytokine profiles observed in MIS-C and pre-pandemic KD are different and might explain the different clinical patterns; 2) SARS-CoV-2 specific immune-complexes (IC) may explain the immunopathology of MIS-C.

Patient's blood samples were drawn prior to any treatment for the quantification of 34 circulating cytokines and evaluation of the presence of circulating SARS-CoV-2 IC. 56 patients were included: 1) 14 with MIS-C (8 positive for SARS-CoV-2 by PCR or serology); 2) 10 with positive PCR to SARS-CoV-

2 without MIS-C (COVID); 3) 14 with pre-pandemic KD and 4) 20 pediatric healthy controls (HC; with negative SARS-CoV-2 IgG/IgM/IgA).

Compared to HC, MIS-C and KD groups displayed significant higher levels of most cytokines, ranging from 1-6 Log_2FC (Log_2 Fold Change). Of these, IFN-γ-related (IL-18, IFN-γ, IP-10) and inflammatory monocytes-related cytokines (MCP-1, MIP1-β, IL-1α, IL-1RA), were the main triggers of inflammation. Through linear discriminant analysis, MIS-C and KD profiles were not distinguishable; however, a subgroup of MIS-C patients differentiates from KD with a marked increase of selected cytokines (IL-8, IL-18, IFN-γ, GM-CSF and RANTES). There was no detection of circulating SARS-CoV-2 IC in MIS-C patients.

Our findings suggest a major role of IFN-γ in the pathogenesis of MIS-C, which can be of relevance for the therapeutic management of affected patients.

F452. : Characterization of epitope repertoire in SARS-CoV-2+ patients

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As COVID-19 has emerged as a global pandemic, a comprehensive understanding of the antibody epitope repertoire will play a significant role in the development of diagnostics, prognostics, vaccines, and therapeutics. We present results from the application of serum epitope repertoire analysis (SERA) to 812 samples from 585 COVID patients relative to our database of thousands of pre-pandemic controls. Since SERA is based on a bacterial display library with randomized peptides, signal can be detected against an arbitrary proteome, including in retrospective, pre-pandemic samples. We identify epitopes and motifs that are both sensitive and specific to SARS-CoV-2 NAT+ patients. Leveraging these motifs, we develop a panel with ~80% sensitivity and ~99% specificity across multiple cohorts. Within these motifs, we collate clusters of patients with different dominant reactivities, including nucleocapsid IgM+, spike glycoprotein IgM+, and broadly IgG+/IgM+. In patients with known disease severity, we identify epitope subsets that distinguish mild and moderate/severe patients with high accuracy. Patient-specific CoV-2 epitopes were observed within the receptor binding domain (RBD) of spike glycoprotein, although few were conserved. Of the identified epitopes, some are specific to SARS-CoV-2 and others are conserved across the proteomes of multiple, common coronaviruses. Collectively, our results provide a nuanced understanding of the SARS-CoV-2 epitope repertoire with potential utility in the development of diagnostics/prognostics, vaccines, and antibody therapies.

F665. Assessment of SARS-CoV-2 Specific CD4(+) and CD8(+) T Cell Responses Using MHC Class I and II Tetramers

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The success of SARS-CoV-2 (CoV-2) vaccines is measured by their ability to mount immune memory responses that are long-lasting. To achieve this goal, it is important to identify surrogates of immune

protection, namely, CoV-2 MHC Class I and II immunodominant pieces/epitopes and methodologies to measure them. Here, we present results of flow cytometry-based MHC Class I and II QuickSwitch™ platforms for assessing SARS-CoV-2 peptide binding affinities to various human alleles as well as the H-2 Kb mouse allele. Multiple SARS-CoV-2 potential MHC binders were screened and validated by QuickSwitch testing. While several predicted peptides with acceptable theoretical Kd showed poor MHC occupancies, fourteen MHC class II and a few MHC class I peptides showed promiscuity in that they bind with multiple MHC molecule types. With the peptide exchange generated MHC tetramers, scientists can assess CD4+ and CD8+ immune responses to these different MHC/peptide complexes. Results obtained with several SARS-CoV-2 MHC class I and II peptides in MHC binding assays and tetramer T cell stainings are included and discussed.

TH343. A multiplex, Luminex-based assay for the detection of antibodies directed against SARS-CoV-2 Proteins

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Determining the status of antibodies specific to SARS-CoV-2 is important for clinical care and epidemiological purposes in the era of the COVID19 pandemic. This study describes the development and validation of a high throughput multiplex Luminex antibody detection assay. The Luminex platform and assay configuration are essentially identical to the single antigen bead assays already used in the majority of histocompatibility laboratories around the world and could easily be implemented into routine screening of transplant candidates and recipients. This novel assay has the capacity to simultaneously identify patient responses to five distinct regions of SARS-CoV-2 proteins. These responses include detection of antibodies specific to the full spike protein, the S1 domain, the S2 domain, the ACE-2 receptor binding domain and nucleocapsid. Antibody responses to these proteins are SARS-CoV-2 specific as antibodies against four community coronaviruses do not cross-react. Specificity and sensitivity are 98.6% and 100%, respectively. Most patients with confirmed SARS-CoV-2 infection generate antibodies to all five specificities, with a minority having antibodies to only a subset of these. Ongoing work to expand the antibody targets, functional consequences of these antibodies and to determine clinical correlations of the different specificities will be presented. This new assay provides a novel and valuable tool to interrogate the spectrum of immune responses to SARS-CoV-2 and is uniquely suitable for use in the transplant setting as well as for research purposes to better understand the immune response to this unique viral pathogen.

TH345. FAIR Data Curation Promotes Rapid Response to COVID-19: The iReceptor Project promotes data reuse by providing access to over 700M antibody/B-cell and T-cell receptor sequences from COVID-19 patients

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AIRR-seq-data (antibody/B-cell and T-cell receptor sequences from Adaptive Immune Receptor Repertoires) can describe the adaptive immune response to SARS-CoV-2 infection in exquisite detail, and comparison and analysis of these data across studies and institutions can greatly contribute to the development of anti-COVID diagnostics and therapeutics, including vaccines.

The AIRR community has developed protocols and standards for curating, analyzing and sharing AIRR seq data (www.airr-community.org) including the AIRR Data Commons, a set of geographically distributed repositories following the AIRR Community's metadata standards. The iReceptor Gateway (gateway.ireceptor.org) is designed to query the AIRR Data Commons for specific "metadata", e.g. "find all repertoires from studies of ovarian cancer" or for specific sequence annotation features (e.g. CDR sequences). The Gateway then aggregates these repertoires from multiple repositories for further analysis by sophisticated AIRR-seq algorithms.

Recently the AIRR community called for increased data sharing to help overcome the COVID-19 pandemic. Many COVID-19 researchers have responded, even providing data from studies during pre-print stage. By mid-September the AIRR Data Commons includes >700M AIRR-seq sequences from 17 studies of COVID-19 patients all curated according to the AIRR Community Standards.

Initial analyses from these studies indicate shared receptor sequences, restricted V gene usage, and characteristic patterns of TCR and BCR clonal expansion among AIRR-seq repertoires from COVID-19 patients. The ability to confirm such patterns by comparing results across studies and institutions will be greatly facilitated by integrated searches across the AIRR Data Commons through the iReceptor Gateway. For more information on obtaining or sharing COVID-19 data contact support@ireceptor.org.

TH351. Prognostic Value of Selective Biomarkers in Non-Severe Versus Severe COVID-19 Cases

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The dramatic increase of COVID-19 patients has created a worldwide health emergency due to its high infectiousness and high rate of mortality in critically ill patients. Therefore, early recognition of disease severity is essential for timely triaging of patients, and the commencement of optimum management that will result in a low mortality rate.

During hospital admission, the initial triage to determine disease severity is largely based on the clinical status of concurrent comorbidities and peripheral oxygen saturation (non-severe ≥ SpO2 94%; severe <

SpO2 94%) levels of COVID-19 patients. In addition, the level of biomarkers such as C-reactive protein (CRP), D-dimer, Ferritin in association with low lymphocyte count has been considered, as a mean of risk stratification.

A limited number of studies have suggested that an increase in D-dimer blood levels and a decrease in CRP blood levels over time may be used as a predictor of pulmonary embolism, which is one of the primary causes of mortality in COVID-19 patients. Furthermore, observation from an unpublished study suggests increasing CRP level is one of the earliest biomarker changes in the blood that reflects physiological alternation.

In light of this context, this single-center, retrospective, observational study in Dhaka, Bangladesh explores the prognostic value of CRP, D-dimer, Ferritin in the assessing degree of severity among thirty COVID- 19 patients grouped into non-severe and severe cases.

Results suggest that the prognostic value of these biomarkers can be considerably used in distinguishing disease severity and predicting adverse outcome in patients with COVID-19.

TH355. An ELISA Protocol with Resolution at High Sample Concentration Reveals Reactive Antibodies to SARS-CoV-2 in Unexposed Individuals

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The SARS-CoV-2 pandemic severely impacted our way of life in 2020. A serology test that can detect lower levels of virus-reactive antibodies than current assays could improve our understanding of pre-existing cross-reactive immunity and SARS-CoV-2 transmission and pathogenesis. To improve the sensitivity of SARS-CoV-2 antibody detection, we developed a new ELISA protocol with a distinct plate washing procedure that minimizes well cross-contamination. Results with this protocol (the 'BU ELISA') show very low signal from plasma or serum samples added to uncoated wells at as low as a 1:5 dilution, enabling visualization of lower quantities of virus-specific IgM, IgG, and IgA antibodies than could be previously detected. Use of this method revealed circulating antibodies that react with recombinant SARS-CoV-2 receptor binding domain (RBD) and nucleocapsid protein (NP) in samples collected before December 2019 in 48 and 96 percent of individuals tested, respectively, with no SARS-CoV-2 RBD-reactive IgG antibodies found in subjects over 70 years old. Among pre-pandemic samples, SARS-CoV-2 NP-reactive antibodies were present at similar levels to SARS-CoV-2 infected subjects in some subjects and very low in others. Also, samples drawn in May 2020 from two individuals with no symptoms or no known virus exposure contained SARS-CoV-2 RBD-reactive antibodies at intermediate amounts compared with other subject groups (higher than pre-pandemic and lower than SARS-CoV-2 infected). We propose that this ELISA protocol, which is straightforward to perform, low cost, and uses

readily available commercial reagents, could be a useful tool to elucidate new information about SARS-CoV-2 transmission, prevalence, and immunity.

TH377. Underrepresentation of South America HLA frequencies affects peptide-based SARS-CoV-2 control strategies

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SARS-CoV-2 is responsible for the COVID-19 pandemic. HLA-epitope interaction is a key element of the humoral and cellular immune response, playing an important role in anti-COVID-19's strategies. Therefore, current control efforts include the development of peptide-based vaccines and diagnostic tests. This requires knowledge of the HLA allele diversity. However, the largest allele repository (Allele Frequency Net Database - AFNDB) contains little information about South America, resulting in underrepresentation. To address this, we performed an extensive collection of datasets available in scientific literature and/or in the AFNDB. We gained more than 12 million datapoints and updated the known HLA allele frequencies. Then, using the most frequent alleles per country (frequency≥5%), we predicted epitopes in SARS-CoV-2 proteins. Candidate epitopes were selected based on their binding to South American HLA alleles. Class-II epitopes were also selected by their structural accessibility. We obtained candidate epitopes with reported experimental evidence in other coronaviruses (14 class-I and 4 class-II), as well as 13 HLA-I and 30 HLA-II novel candidates.

Similar studies have reported SARS-CoV-2 peptides attempting global coverage. They used either the most frequent HLA supertypes or IEDB's population tool, which uses AFNDB's HLA frequencies information. Here, we show that these peptides poorly cover the most frequent South American HLA alleles, while our candidate epitopes cover all of them. Finally, updated HLA frequencies will allow a better representation of South America in different immunogenetic studies beyond SARS-CoV-2, being potentially useful to study other infectious and autoimmune diseases, as well as cancer predisposition, treatment, and anti-tumor immunity.

TH398. Induction of a regulatory myeloid program in bacterial sepsis and severe COVID-19

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A recent estimate suggests that one in five deaths globally are associated with sepsis. To date, no targeted treatment is available for this syndrome, likely due to substantial patient heterogeneity and our lack of insight into sepsis immunopathology. These issues are highlighted by the current COVID-19

pandemic, wherein many clinical manifestations of severe SARS-CoV-2 infection parallel bacterial sepsis. We previously reported an expanded CD14+ monocyte state, MS1, in patients with bacterial sepsis or non-infectious critical illness, and validated its expansion in sepsis across thousands of patients using public transcriptomic data. Despite its marked expansion in the circulation of bacterial sepsis patients, its relevance to viral sepsis and association with disease outcomes have not been examined. In addition, the ontogeny and function of this monocyte state remain poorly characterized. Here, using public transcriptomic data, we show that the expression of the MS1 program is associated with sepsis mortality and is up-regulated in monocytes from patients with severe COVID-19. Furthermore, we show that blood plasma from bacterial sepsis or COVID-19 patients with severe disease induces emergency myelopoiesis and expression of the MS1 program, which are dependent on the cytokines IL6 and IL10. Finally, we demonstrate that MS1 cells are broadly immunosuppressive, similar to monocytic myeloid-derived suppressor cells (MDSCs), and have decreased responsiveness to dsRNA stimulation. Our findings highlight the importance of regulatory myeloid cells in sepsis prognosis and the role of systemic cytokines in inducing emergency myelopoiesis during severe infections.

TH399. Plasma proteomics of SARS-CoV-2 infected patients reveals insights into pathways mediating disease severity, immune aging, and neutralization

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As of 8/2020, COVID19 has caused over 600,000 deaths globally; however, cellular mechanisms underlying the host immune response are poorly understood. To investigate the systemic immune and tissue-related effects of SARS-CoV-2, we analyzed 1436 plasma proteins in 306 SARS-CoV-2 patients and 78 symptomatic controls over serial timepoints. Over 600 differential plasma proteins were associated with SARS-CoV-2 infection, punctuated by a viral response signature (higher expression of DDX58, IFNg, IFNL1, CXCL10, CXCL11). We identified 250 differential plasma proteins in severe versus non-severe disease, including persistently higher levels of IL6, IL8, IL24, IL1RL1, CXCL10, CCL2, CLL7, CCL8 and CCL20 in those who died. We analyzed 5 published scRNAseq datasets and found that monocytes, neutrophils and plasmablasts were the primary peripheral immune contributors for severity-associated protein signatures. Within lung tissue, severity-associated proteins were expressed in lung epithelial, AT1, AT2, goblet and ciliated cells, and tissue-resident immune cells including monocytes, macrophages, CD4 and CD8 T cells. This highlights a potentially unique contribution of tissue-resident T cells versus peripheral T cells to the plasma proteome. We found high neutralization titres correlated with signatures of plasma cells (MZB1, SDC1) and Th2 response (CD40LG, TNFRSF4, CCL17, CCL18), and negatively correlated with patient age, monocytic response (SIGLEC1, CCL8, CD300E, TNFSF13B, CXCL10), and T cell exhaustion (LAG3) and suppressive (GPA33) phenotypes. Our data highlight the use of plasma proteomics to dissect peripheral and tissue-

specific immune responses to SARS-CoV-2 and uncover mediators of severity and neutralization in the setting of active viral infection.

TH411. Unravelling immune and cellular responses associated with acute COVID-19 infection, symptoms and lethality at single-cell resolution

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Several SARS-CoV-2 related deaths are due to acute respiratory distress disorder (ARDS), or direct complications thereof. We hypothesize that COVID-19-associated-ARDS develops as a result of a dysfunctional host immune response that contributes to clinical deterioration in the acute phase of systemic illness, leading to ineffective viral clearance and collateral tissue damage. To address this, we collected blood from 80 COVID-19 negative and 306 positive patients that presented at MGH Emergency Department with ARDS. Serial blood samples were collected at day 3 (n=220) and day 7 (n=141) of patient's hospitalization. A total of 747 blood samples spanning the WHO severity spectrum were analyzed through single-cell RNA-sequencing, with paired measurement of 197-surface proteins (CITE-seq) and paired TCR- and BCR-sequencing. Analyses involve mapping activation states and T cells and B cell clones tracking with disease progression, and identify immune cell states and signaling pathways associated with ARDS severity. The analysis of the first 350,000 single cells highlighted two monocyte states significantly associated with worse outcomes, respectively characterized by S-calprotectin (S100A8/A9) and *NEAT1* expression. In contrast, several cell states were detected in significantly increasing proportions associated with better outcomes, including homing naïve $CD4^+$T cell (*CCR7*), cycling $CD8^+$ T cells, *TCL1A*-expressing B cells, and conventional dendritic cells. We will present detailed analyses of all samples. Collectively, this study analyzes one of the largest COVID-19 acute cohorts, with potential to identify new therapeutic and biomarker targets for COVID-19 associated ARDS.

Genetics and Epigenetics

F175. Immunomodulatory Activity of Epigenetic Drugs Combinations in Mesothelioma: Laying the Ground for New Immunotherapeutic Strategies

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Growing evidence are showing the efficacy of immunotherapy in malignant mesothelioma (MM). However, during MM progression, epigenetic changes negatively regulate the cross-talk between tumor cells and immune system, that nevertheless could be reprogrammed by epigenetic drugs. In this study, we compared the immunomodulatory potential of the DNA hypomethylating agent guadecitabine with the histone deacetylases inhibitor valproic acid (VPA), the EZH2 inhibitor EPZ-6438, alone or combined with guadecitabine, in 4 MM cell lines. We evaluated the expression of HLA class I molecules by flow-cytometry and of PD-L1, cancer-testis antigens (CTA: NY-ESO, MAGE-A1), Natural Killer Group-2D-Ligands (MIC-A, MIC-B, ULBP2), and cadherins (CDH1, CDH2) by qRT-PCR. Guadecitabine or VPA upregulated the expression of HLA class I antigens (mean fold-change, FC_m=1.75 or 1.67), PD-L1 (FC_m=2.38 or 3.17), MIC-A (FC_m=1.96 or 1.78), MIC-B (FC_m=2.57 or 3.04), ULBP2 (FC_m=3.56 or 3.75). Guadecitabine upregulated/induced CTA expression in all cell lines and VPA upregulated CTA expression in 1 cell line. EPZ-6438 up-regulated NY-ESO-1 and MIC-B expression in 1 cell line. Combination of VPA or EPZ-6438 with guadecitabine strengthened their effects, upregulating HLA class I antigens (FC_m=2.55 or 2.69), PD-L1 (FC_m=8.04 or 2.65), MIC-A (FC_m=3.81 or 2.26), MIC-B (FC_m=8.00 or 3.03), and ULBP2 (FC_m=6.24 or 4.53) expression. High levels of CTA expression were observed after combination treatments vs. guadecitabine. CDH1 expression was induced in 2 sarcomatoid cells by guadecitabine alone or *plus* VPA/EPZ-6438; CDH2 expression was upregulated by VPA alone (FC_m=1.50) or *plus* guadecitabine (FC_m=2.54). Overall, combination strategies of selected epigenetically-based immunotherapies could be effectively pursued in MM clinic.

F225. Analysis of Molecular Pathways Identified from Single Nucleotide Polymorphisms Demonstrates Mechanistic Differences in Systemic Lupus Erythematosus Patients of Asian and European Ancestry

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Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disorder with a prominent genetic component. Evidence has shown that individuals of Asian-Ancestry (AS) experience the disease more severely, exhibiting increased renal involvement and tissue damage compared to European-Ancestry (EA) populations. However, the mechanisms underlying elevated risk in this population remain unclear. Here, we applied a comprehensive systems biology approach using single nucleotide polymorphisms (SNPs) associated with SLE in patients of Asian and European ancestry to identify molecular pathways

associated with disease pathogenesis. Using 2108 SLE SNPs from transancestral Genome Wide Association studies, we identified 4350 ancestry-specific and trans-ancestry potential genetic drivers of SLE (2497 Asian, 1696 European and 157 shared). This includes all eQTLs, genes affected by altered transcription factor binding sites, those with amino acid substitutions and genes most proximal to the SNP. The very low percentage of shared genetic drivers (3.7%) was notable. Gene associations were linked to upstream and downstream regulators using connectivity mapping and the resulting sets of predicted SLE-associated biological pathways were mined for candidate drug targets. Importantly, pathways driven by Asian-associated genes were enriched in processes related to metabolism, proliferation and cell stress, whereas European-associated genes were enriched in immune-based processes, including cytokine signaling, pattern recognition receptor signaling and T and B cell signaling. Shared pathways included TH1 and TH2 activation.Together, these analyses suggest fundamental differences in SLE-risk related molecular pathways, the majority of which are linked to ancestral differences, and suggest novel drug candidates that might differentially impact Asian and European individuals with SLE.

F299. A Web-Based Immune Phenotype Reference from Harmonized RNA-seq Data

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RNA sequencing is now a standard scientific tool for transcript profiling in immunologic research. It is used to assess gene expression, define biomarkers, and deduce cell heterogeneity. Generating human RNA-seq datasets is often expensive and sometimes it is not possible to collect data from healthy controls. Currently there is no online resource where scientists can survey aggregated RNA-seq data tailored specifically for immunology. We developed an interactive web-based reference for scientists to explore the immune-landscape using RNA-seq data from over 300 healthy control subjects. We aggregated ten open-access studies from the National Institute of Allergy and Infectious Disease (NIAID) funded repository, ImmPort (immport.org). This produced a reference for three common immunologic samples- whole blood, peripheral blood mononuclear cells, and sorted T cells. Control subjects span all ages, from newborn infants to the elderly, and come from all regions of the world. The web interface for our platform allows researchers to swiftly generate their own graphs and datasets from any device with internet. Graphs are interactive, allowing users to subset data based on sex, ethnicity, and age. Deconvolution algorithms were used to produce graphs of not just gene expression, but also cell composition phenotypes. We applied rigorous non-Gaussian statistical methods to harmonize data. These techniques account for both batch effect and the skewed nature of RNAseq. This research is part of the larger 10k Immunomes project (10kimmunomes.ucsf.edu), a resource of over 10,000 control subjects with multiple immune measurements derived from the ImmPort database.

F300. Differences in transcriptional signatures between Asian and Caucasian systemic lupus erythematosus patients from sorted cells.

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Systemic lupus erythematosus (SLE) is a progressive chronic autoimmune disease in which outcomes vary among different ethnic groups. Little is known about why individuals might develop a particular SLE phenotype. To further understand these ethnic differences we performed cell-specific transcriptome analysis from patients enrolled in The California Lupus Epidemiology Study (CLUES), a well-characterized longitudinal multiethnic cohort.

RNA-Seq data for 4 immune-cell types (monocytes, B cells, CD4-T cells and NK cells) has been generated for 120 patients (63 Asian and 57 White individuals) of the CLUES cohort to study the specific-transcriptomic remodeling in each of these cell types between individuals of Asian or White ethnicity.

Principal Component Analysis (PCA) revealed separation by cell type and not by ethnicity. Although we don't observe global changes associated with ethnicity, differentially expressed genes (FDR< 0.05 and log2FC 0.5) between Asian and Caucasian groups have been identified for each cell-type. Monocytes and CD4-T cells present with an enrichment of significantly up-regulated genes in the Asian population with up/down 276/35 and up/down 82/10 genes respectively, whereas B cell and NK cells are enriched for up-regulated genes in Caucasians (B cell: up/down 257/34; NK cells: up/down 67/0). Pathway analyses revealed enrichment of metabolism and transcriptional activity in monocytes and CD4-T cells whereas both B cell and NK cells are enriched for integrin and IL2 signalling pathways indicating a higher activity of specific cell types in Asians and Caucasians respectively.

These results suggest a cell-specific transcriptional signature expression in SLE patients of ethnically different background.

F412. Exploring Post-translational Histone Modifications of Immune Cells Using EpiTOF and EpiAtlas

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Post-translational modifications (PTMs) of histrones are a key regulator of many biological processes. However, their role in the immune system has been largely unexplored, due to lack of data at single-cell resolution. We recently described EpiTOF, a high-throughput, multiplexed, mass cytometry-based method for measuring histone PTMs at a single-cell level. Here, we described EpiAtlas, the largest database to date of 39 histone PTMs in >54 million cells across 28 immune cell types from 129 healthy individuals profiled using EpiTOF. Using EpiAtlas, we identified epigenetically distinct subsets of individual immune cell types. We also leveraged the large number of samples in EpiAtlas to identify robust changes in histone PTMs over aging in specific immune cell types – finding that histone modifications do not change in a continuous, linear fashion and have certain conserved patterns across different modifications and cell types. We also identify age-matched, immune cell-specific healthy

histone PTMs profiles that can be used as reference for epigenetic profile of healthy immune cells. Finally, we demonstrate hitherto unprecedented opportunity presented by EpiAtlas by investigating single-cell histone PTM profiles of hematopoietic progenitors, a rare cell type extremely difficult to profile using ChIP-seq, and demonstrate the epigenetic changes progenitors exhibit throughout differentiation. Using EpiAtlas, we are able to uncover biology at a single-cell level previously unknown.

F420. Waddington landscape of healthy human PBMCs at single cell resolution reveals global cellular methylations at the crossroad of immune cell life span and memory

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The histone language has been mostly studied *in vitro* or lower eukaryotes using direct mutagenesis of sites of histone modifications. Similar experimental designs are more difficult to obtain *in vivo* and higher eukaryotes, if not impossible in humans. Here we present the first single-cell resolution systems biology study of complex and nuanced crosstalk between histone PTMs i.e. histone language. We leveraged computational approaches and EpiTOF - a high-throughput mass cytometry technology that easily profiles several millions of cells and up to 40 histone PTMs and histone variants. EpiTOF consists of two panels, one mainly measuring selected histone methylations, and another selected histone acetylations, phosphorylations, and ubiquitinations at a single-cell level. Both panels share phenotypic cell surface markers for immune cells of human peripheral blood.

We profiled 27,841,803 peripheral blood mononuclear cells (PBMCs) for 37 histone PTMs at a single-cell resolution across 5 independent cohorts consisting of 65 healthy individuals. The wealth of data at a single-cell resolution presents a novel opportunity to test whether interactions between histone PTMs constitute a regulatory network, which can potentially reveal novel PTMs interactions i.e. the histone language.

Here we describe (1) a comprehensive epigenetic network of histone PTM interactions in human PBMCs, (2) novel epigenetic associations between a decrease in global cellular methylations, immune cell life span and memory, and (3) a framework to read the histone language through computational modeling of differentiation of human monocytes in the blood.

TH2. Single cell transcriptomic analysis for a better understanding of human CD8+ regulatory T cells

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Strategies based on regulatory T cells (Tregs) are promising to prevent graft rejection. Our group has shown that rat and human $CD8^+CD45RC^{low/-}$ Tregs display significant suppressive function. We have also shown that cell therapy using human $CD8^+CD45RC^{low/-}$ Tregs was efficient to prevent graft rejection and GVHD in humanized NSG mice models. However, the heterogeneity of the $CD8^+CD45RC^{low/-}$ Tregs population is important from a phenotypic point of view, suggesting that either a fraction of the population is tolerogenic, or the induction of tolerance is due to a combination of cells forming an "immunological niche". To discriminate between these two hypotheses, we sorted $CD8^+CD45RC^{low/-}$ Tregs from blood of healthy volunteers and sequenced their transcriptomes by single cell RNA-seq methods. The cell transcriptomes analysis highlighted the heterogeneity inside the population with the identification of 4 distinct clusters with a specific signature. We identified one cluster of particular interest based on its immune and regulatory gene signature. However, other genes such as *TGFβ* were expressed by all clusters inside $CD8^+CD45RC^{low/-}$ T cells. We then focused our analysis on genes that encode for membrane proteins that have never been used for $CD8^+$ Tregs isolation yet such as TNFR2 and ITGB1. We confirmed their expression at protein level by a subset of $CD8^+CD45RC^{low/-}$ T cells.
In conclusion, this project characterized the heterogeneity inside $CD8^+CD45RC^{low/-}$ T cells. It will help us to better define a consensus phenotype for $CD8^+$ Tregs and will improve the use of human $CD8^+$ Tregs as a cell therapy for treating transplanted patients.

TH18. The thymic hypoplasia in 22q11.2 deletion syndrome (DiGeorge syndrome) results from an inability of mesenchymal cells to support tissue expansion

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BACKGROUND. 22q11.2 deletion syndrome (22q11.2del) is the most common human chromosomal disorder known (1/4000). 22q11.2del patients have congenital malformations that can include a thymic hypoplasia, which leads to a T cell lymphopenia. The molecular cause of the thymic hypoplasia is unknown, although it occurs among the stromal cell populations.

OBJECTIVE. To determine which stromal cell populations are causal to the thymic hypoplasia in 22q11.2del (DiGeorge) patients.

METHODS. Mouse models of 22q11.2del are used to analyse the specification and expansion of the thymus during embryogenesis. Hypoplastic thymic lobes are isolated and characterized by flow

cytometry and compared with normal tissues. The stromal cell populations and hematopoietic cells from normal and hypoplastic embryonic thymic lobes are flow sorted, mixed in various combinations, and used in reaggregate thymic organ cultures (RTOC). Single cell RNA-seq is used to identify transcripts necessary for thymic tissue specification and expansion.

RESULTS. Flow cytometric analyses of normal and hypoplastic thymic lobes from e13 embryos revealed similar percentages of mesenchymal, epithelial, and thymic progenitors. In RTOC, replacing the 22q11.2del-derived mesenchymal cells with those from normal fetal thymuses restored tissue expansion and thymopoiesis. 10X single cell RNA has revealed key transcripts necessary for thymic tissue expansion.

CONCLUSION. The thymic hypoplasia resulting from 22q11.2del syndrome is caused by mesenchymal cell defects. Importantly, replacing the 22q11.2del mesenchyme with those from normal thymuses enables thymic tissue regeneration and expansion. This could yield new clinical strategies for correcting the thymic hypoplasia in DiGeorge patients.

TH49. Integrative functional interrogation on a CDKN1B variant defining the mechanistic underpinnings of target genes for lupus susceptibility

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A recent genome-wide association study reported a significant genetic association between rs34330 (-79C/T) of cyclin-dependent kinase inhibitor 1B (*CDKN1B*) gene and risk of systemic lupus erythematosus (SLE) in Han Chinese population. However, its validity and functional mechanisms of action to SLE susceptibility are not yet defined. Here, we performed an allelic association followed by a meta-analysis using 11 independent cohorts (n=28,828), *in-silico* bioinformatics, and a series of experimental validations to determine the functional consequence of rs34330. We first replicated the genetic association with rs34330 (P_{meta}=1.48 x 10^{-20}, OR=0.84). Following-up with bioinformatics and eQTL analyses, we predicted the rs34330 is located in an active chromatin region that could regulate promoter and/or enhancer activities of the target gene(s). Using luciferase, we observed significant allele-specific promoter activity in HEK293 (kidney) and Jurkat (T-cells). Using ChIP-qPCR, we found allele-specific bindings with three histone marks (H3K27Ac, H3K4Me3, and H3K4Me1) and two transcription factors (Pol-II and IRF-1). Next, we experimentally validated the long-range chromatin interactions between rs34330 and target genes using chromosome conformation capture (3C) assay. Finally, applying CRISPR-based genetic and epigenetic editing, we confirmed the regulatory role of this region containing rs34330 for *CDKN1B* and nearby gene (*APLOD1* and *DDX47)* expressions. Taken together, we replicated genetic association between a potentially functional variant and SLE. The risk allele (C) is likely to influence binding affinities with several histones and regulatory proteins (i.e., IRF1) to regulate the allele-specific expressions of target genes *(CDKN1B/APOLD1/DDX47)*. Hence, their aberrations could be a potential mechanism for exacerbating lupus susceptibility through rs34330.

TH128. Immune cell promoter connectomes reveal lineage-specific gene regulatory architectures and suggest mechanistic bases of genetic susceptibility to autoimmune disease

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Genome-wide association studies (GWAS) have identified hundreds of loci associated with susceptibility to autoimmune disease, but the causal variants, the genes involved, and the tissues impacted are unclear. We present a systematic analysis of the expression and physical connection of genes to autoimmune disease variants in multiple human cell types (monocytes, germinal center B cells, follicular helper T cells, Th1 cells, Th2 cells, Th17 cells, and Treg) using high-resolution, promoter-focused Capture-C coupled with ATAC-seq and RNA-seq. We identified lineage-specific *cis*-regulatory architectures across >20,000 genes in each cell type, revealing the structural basis of cell-specific control on gene transcription. The resulting disease-associated 'variant-to-gene' maps connected 1850 variants at over 50% of autoimmune GWAS loci to 2,590 putative effector genes enriched for functional categories such as interferon signaling and T helper differentiation. Approximately half of the target genes implicated by these spatial gene regulatory maps are specific to a single disease, such as *DDX6* for celiac and *STAT6* for psoriasis. The other half (*ATG16L1, REL, IKZF3*) were shared by multiple autoimmune diseases. Interestingly, open chromatin regions harboring a single disease variant were commonly found connected to different genes in different cell types (e.g., T1D proxies at '*GSDMB*' contact *LRRC3C, IKZF3* and *ERBB1* in all lymphocytes, while connecting specifically to *THRA* in TFH), suggesting various mechanisms by which the impact of genetic variation is compounded and diversified across multiple tissues and gene regulatory networks. These 3D disease-associated gene regulatory architectures provide a rich resource for understanding the genetic basis of autoimmune disease susceptibility.

TH330. Detection of Immunosenescent T Cell Gene Signature in Alzheimer's Disease Subjects

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Aging is the main risk factor for Alzheimer's disease (AD) that exerts profound impacts on the immune system. The role of T cells in AD and how it relates to neuroinflammation is not clear. Using RNA sequences of four CD3+ T cell subsets (naïve and memory CD4+ and CD8+ T cells) from the blood of AD and control age- and sex-matched subjects (n=100) of the Rush Memory and Aging Project (MAP), we built a molecular network using modules of coexpressed genes and then related these modules to AD and its neuropathologic and cognitive endophenotypes. We identified sets of immunosenescent

genes in CD4+ and CD8+ T cells that are associated with clinical AD, neurofibrillary tangles or cognitive decline. Interestingly, module 1 is enriched for the gene network associated with IL-10 signaling in CD4+ T cells, whereas module 3 is associated with cytotoxic effector genes, including granzymes A, H and K, in CD8+ T cells. Next, we assessed post-mortem brains of AD and control individuals (no neurological disease) using immunohistochemistry to examine the expression of CD3 and amyloid deposition. We found numerous extravascular CD3+ T cells in the perivascular space of blood vessels with cerebral amyloid angiopathy in the hippocampi of AD brains, but not in control subjects. Our study provides a direct link between adaptive immune T cells and AD pathology. Ongoing studies are focused on dissecting the role of the AD-associated T cell senescent molecules *in vivo* using animal models of AD.

TH439. Coronin1A heterozygosity and compound heterozygosity of pathogenic ZAP70 mutations in a young child with severe combined immunodeficiency

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We report a male term infant, first-born to non-consanguineous parents of British ancestry who presented at two months of age with respiratory failure, profound failure to thrive, chronic Rotavirus shedding (presumed vaccine strain) and severe eczema. There was no family history of immune deficiency. The baby was found to be cachectic, with extensive rash, sparse hair, generalised lymphadenopathy and soft dysmorphic facial features, and required mechanical ventilatory and intensive level care support.

Complete blood count was unremarkable but on subset analysis, CD8+ T cells were almost absent (CD3 3113/ul, CD4 2899/ul, CD8 100/ul, CD19 1609/ul, NK 205). Thymic shadow was present, and an extensive workup for infections including opportunistic pathogens negative. Initial microarray analysis showed heterozygosity for a 0.9Mb 16p11.2 deletion involving CORO1A, with maternal inheritance. Homozygosity of this defect has been linked with autosomal recessive immunodeficiency, resulting in a rare form of severe combined immunodeficiency. Panelled exome sequencing of 350 immunodeficiency genes confirmed heterozygosity for CORO1A microdeletion, but identified additional compound heterozygosity of pathogenic ZAP70 mutations. Following conditioning with Alemtuzumab, Treosulfan and Fludarabine, the child received a 10/10 matched, peripheral blood stem cell transplantation, aged 6 months. At six weeks post-transplant he displays 100% donor chimerism, without signs of graft versus host disease.

This is an instructive case, illustrating compound heterozygosity of ZAP70, including a previously not described pathogenic polymorphism, in combination with heterozygosity for a CORO1A microdeletion. Hypomorphic CORO1A mutations have been identified in patients with a multitude of immunologic abnormalities, and may have impacted the presenting phenotype.

Human B cells and Antibodies

F13. Human adipose tissue-derived antibodies have “anti-self” specificity and an unusual mechanism of stimulation

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Our laboratory has previously shown that human obese adipose tissue (AT) secretes adipocyte-specific IgG antibodies without any exogenous stimulation. The ongoing process of cell death in the obese AT likely leads to release of “self” antigens able to induce chronic stimulation of B cells. Here we confirm and extend our initial observation on a different cohort of individuals and also show that the plasma of obese individuals is enriched in IgG antibodies with specificities for adipocyte-derived antigens. Adipocyte-specific IgG secreted by obese AT are significantly correlated with those present in plasma.

Using immunoprecipitation and mass spectrometry, we have identified these antigenic specificities. The antigens are almost exclusively intracellular or cell-associated, usually not classical “self” antigens, but are released by cells dying in the AT.

These antigens will be used in protein arrays to screen plasma from obese individuals and also those with autoimmune diseases. Our preliminary evidence shows that the plasma of Rheumatoid Arthritis patients is enriched in adipocyte-specific IgG antibodies

Finally, we show for the first time that not only macrophages but also adipocytes in the obese AT are efficient antigen-presenting cells and stimulate the secretion of IgG autoimmune antibodies. They do so because they express the antigen-presenting molecules CD1d and, to a lesser extent, MHC class II, as well as the co-stimulatory molecule CD86. These results may lead to the development of novel therapeutic strategies to control autoimmunity.

F145. Serum IgG Profiling Healthy 1- and 2- Year Old Toddlers Reveals some with Clinically Informative Reactivities to Pathogens and Autoantigens

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BACKGROUND. The antibody repertoire in an infant/toddler develops in response to the microbiome, infections, environmental exposures, and vaccinations. Monitoring the specificity of these antibody responses in normal toddlers will provide indicators of disease susceptibility.

OBJECTIVE. To determine whether profiling of the serum antibody specificities in healthy toddlers provides key insights into the formation of immune response to pathogens and autoantigens.

METHODS. The serum IgG and IgM antibody reactivity patterns in healthy toddlers against infectious agents, autoantigens and vaccine antigens was done with an antigen array. Repeat profiling was performed at year 2 to reveal longitudinal changes in the IgG and IgM responses. Clinical information, DNA sequence polymorphisms, and selected cytokine assays were used to establish an odds ratio for immune disease potential among the cohort.

RESULTS. Healthy toddlers were clustered into low, moderate, and high Ig responder groups. The high responder group had elevated IgG reactions to selected pathogens and autoantigens. This group (17% of cohort) had high odds ratios with maternal diabetes, age, and a family history of asthma. All toddlers developed strong antibody responses to Measles-Mumps-Rubella vaccines (MMR), but more variation was noted towards other vaccines. The high responder group had DNA polymorphisms linked to enhanced immune responses that correlated with elevated cytokine levels as well as eczema and asthma.

CONCLUSION. A subset of normal “healthy” toddlers has a high potential for immune system abnormalities and autoimmunity based on higher serum antibody responses to pathogens and autoantigens, genetic polymorphisms, and elevated cytokine responses.

F256. Quantitative Assessment of NFkB Transcription Factor Activity in Health and Disease

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Common Variable Immunodeficiency (CVID) is the commonest cause of primary immunodeficiency in adults. Many cases are due to single immune gene defects. Nuclear factor kappa B (NFkB) is an important signalling pathway in immune processes. It comprises canonical and non-canonical components. Single gene defects in the NFkB pathway are described in a number of CVID patients, all of whom have functional abnormalities in mature B cells. How genetic defects in this pathway affect the function of B cells is not well defined. We have a developed a robust method to accurately quantify small differences in the functional activity of the NFkB pathway. We show quantitatively that the function of the NFkB response (canonical and non – canonical) is significantly lower in our cohort of CVID patients compared to healthy controls. This proved to be the same whether or not the patients had a monogenic defect in the NFkB pathway itself.

F295. Bregs induce reduced IFN-γ production upon mycobacterial challenge: implications for cord blood transplantation

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Objectives: Umbilical cord blood (UCB) is considered a safe source for stem cell transplantation. Nevertheless, infection is a major concern. IFN-γ is pivotal in anti-mycobacterial defense, and Breg cells, which are increased in UCB, seem to inhibit IFN-γ production by T cells. We aimed at evaluating anti-mycobacterial responses in UCB compared to adult peripheral blood, and the role of Breg in anti-mycobacterial immunity. Methods: APB mononuclear cells (APBMCs) were stimulated with BCG or PMA-Ionomycin in the presence or absence of Breg and/or Treg cells after cell sorting; cytokine production was evaluated by flow cytometry (IFN-γ, TNF-α, IL-10) or Luminex (IFN-γ, TNF-α, IL-10, IL-6, IL-1β, IL-1RA, IP-10, IL-12p70). Breg cell frequency and anti-mycobacterial responses after whole blood stimulation with BCG (cytokine production and activation markers' expression) were compared between UCB (n=16) and APB (n=17). Results: Breg cell presence in APBMCs culture with BCG led to a diminished IFN-γ production by T cells (p=0.01) and a reduction in overall production of IFN-γ (p=0.003). When comparing UCB and APB responses to BCG, UCB showed decreased CD71 activation marker's expression (p=0.002), and IFN-γ (p< 0.001) and IP-10 (p< 0.001) production. IFN-γ and IP-10 stimulation ratio inversely correlated with Breg cell frequency (p=0.004, r=-0.5; p=0.001, r=-0.65, respectively). Conclusion: We have shown a reduction of anti-mycobacterial responses by Breg cells. Given the increased numbers of Breg cells in UCB, the benefit of this source of progenitors for transplantation by increasing tolerance, may also lead to increased susceptibility to intramacrophagic infections.

F340. Natural Acquisition of Antibody Immunity in Infants at High Risk of Severe Respiratory Syncytial Virus Infections

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Transplacentally transferred antibodies confer protection against respiratory syncytial virus (RSV). These defenses are limited in preterm infants and little is known about the development of natural immunity against this virus in their first year of life. To address this question, we performed systems serology on blood samples from 37 infants at high-risk of severe RSV at the time of discharge home after birth ("preseason") and after the 2018-19 winter season ("postseason"). These subjects were born premature, had chronic lung or congenital heart diseases or other comorbidities. Preseason RSV neutralizing antibody titers correlated with gestational age and postnatal age (spearman rho; p value: 0.74; 0.0002 and -0.64; 0.00005 respectively), consistent with antibodies largely coming from the mother at this stage. Concomitantly, RSV-specific IgG1 largely waned within 20 weeks post-birth. However, this decline was contrasted by an increase in Fcγ receptor binding, antibody-dependent phagocytosis (median fold-change for FcγRIIA binding: 1.7-77.9 for various RSV antigens) and RSV-specific IgM levels ($p < 0.05$ for 3 of the 4 antigens tested) suggesting functional maturation of humoral responses. In contrast, influenza-specific FcγR binding and phagocytosis did not significantly increase and continued to be correlated with IgG titers throughout the winter season. These data suggest that natural acquisition of antibody immunity against RSV, likely due a subclinical exposure to the virus, is common in infants at high-risk of severe infections, whereas the same is not true for influenza. Understanding the impaction of these findings on clinical outcomes can help develop prevention/vaccination programs against common respiratory infections during infancy.

F356. Deviated B cell receptor repertoire associated with infection-related episodes at onset in Myalgic encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a chronic, intractable disorder, characterized by post-exertional malaise, sleep disorders, cognitive impairment, autonomic dysfunctions, etc. The onset is often sudden following infection-related episodes. Outbreaks, including the one after SARS-CoV, is documented. A risk of outbreak following COVID-19 infection is debated. Recent findings have indicated the involvement of neuroinflammation with immune abnormalities in

ME/CFS. ME/CFS patients referred to our hospital (n=106) and age and sex-matched healthy controls (n=25) were enrolled. By flow cytometry, we found an increase of plasmablast in a subset of patients (18%). By applying unbiased next generation sequencing technology, we discovered that several immunoglobulin heavy chain variable region (IGHV) gene family usages were significantly ($p< 0.05$) abundant in ME/CFS patients (n=37) as compared with HC (n=23). The main results were replicated in an independent cohort (n=45). In addition, we confirmed a significantly increased frequency of anti-b adrenergic receptor antibody-positive ME/CFS patients as shown by a German group (Loebel et al. Brain, Behavior and Immunity, 2016). Finally, we found that, among the upregulated IGHV, IGHV3-30/3-30-3 is notable in that 1) more frequent in patients with infection-related episodes at onset, 2) more frequent in patients with short disease duration, 3) correlates with IFN response gene expression in plasmablasts of patients, and 4) more frequent in anti-adrenergic b1/b2 receptor antibody-positive patients. To conclude, BCR repertoire analysis could be developed as a diagnostic biomarker of ME/CFS. It could predict the efficacy of B cell-targeted therapy, which was shown to be effective in a subgroup of patients.

F454. Diversion or double-edged sword? Antibody cross-reactivity in HIV/HCV co-infection

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Investigating the human antibody response to HIV and HCV has led to significant advances towards preventative therapeutics and vaccines against these highly mutable pathogens. Despite the high prevalence of HIV/HCV co-infection, little is known about antibody responses in this context. To address this deficit, we investigated the effect of chronic co-infection with HIV and HCV on the development of virus-specific humoral responses, hypothesizing that chronic HIV/HCV co-infection leads to the development of HIV and HCV cross-reactive antibodies. To investigate this, we used LIBRA-seq (Linking B cell Receptor to Antigen specificity by Sequencing), a technology recently developed in our laboratory that uses DNA-barcoded antigens to map B cell specificity through single-cell next-generation sequencing. We investigated class-switched B cells that recognize viral envelope glycoproteins (HIV Env gp160, HCV E1E2) from a chronically HIV/HCV co-infected donor ~3.59 years post HIV infection (ypi) and identified multiple HIV/HCV cross-reactive B cells. We mapped their specificity to a non-neutralizing epitope on HIV gp41 and a neutralizing epitope on HCV E2 corresponding to antigenic region 5 (AR5) by PDB structural alignment, competition ELISA, overlapping peptide ELISA, and *in vitro* neutralization assay. Importantly, isolated cross-reactive antibodies show extraordinary HCV neutralization breadth, neutralizing 19/19 viruses tested. Additional characterization revealed that HIV/HCV cross-reactive antibodies develop from an HCV-specific lineage that is hijacked after HIV infection, and subsequent somatic hypermutation leads to increased affinity for HIV. This

study is the first to directly demonstrates that antibodies raised against one pathogen can enter the immune response against a different, genetically unrelated pathogen.

F455. Pathogenic monovalent IgG4 autoantibody development in myasthenia gravis requires affinity maturation

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Pathogenic IgG4 autoantibodies in autoimmune myasthenia gravis (MG) are functionally monovalent as a result of Fab-arm exchange. The development of these unique autoantibodies is not well understood. We examined MG patient-derived monoclonal autoantibodies (mAbs), their corresponding germline-encoded unmutated common ancestors (UCA) and monovalent antigen-binding fragments (Fabs) to investigate how antigen-driven affinity maturation contributes to both binding and immunopathology. Mature mAbs, their UCA counterparts and mature monovalent Fabs bound to the autoantigen and demonstrated pathogenic capacity. However, monovalent UCA Fabs still bound the autoantigen but did not have measurable pathogenic capacity. The mature Fabs were characterized by very high affinity (sub-nanomolar) driven by a rapid on-rate and slow off-rate. The affinity of the UCA Fabs was approximately 100-fold less than that of the mature Fabs. Crystal structures of two Fabs shed light on how mutations acquired during affinity maturation may contribute to increased MuSK binding affinity. The effect of the autoantibodies on the low-density lipoprotein receptor-related protein 4 (LRP4)/MuSK interaction – which is necessary for acetylcholine receptor signaling – was investigated to further define the pathogenic mechanisms. These collective findings indicate that the autoantigen drives autoimmunity in MuSK MG through the accumulation of somatic mutations such that monovalent IgG4 Fab-arm exchanged autoantibodies reach a high affinity threshold required for pathogenic capacity.

Immune monitoring

F206. Extensive Profiling of Human Cytomegalovirus-Specific T Cell Phenotypes Prior to Lung Transplantation to Predict Post-Transplant Risk of Rejection

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After transplantation, cytomegalovirus (CMV) can reactivate and cause infection and disease in CMV seropositive solid organ transplant recipients. CMV is also a risk factor for graft rejection. We sought to test whether features of the pre-transplant CMV-specific T cell response could predict risk of post-transplant allograft rejection. Using a highly multiparametric, 37-marker, mass cytometry (CyTOF) panel, we have analyzed the pre-transplant T cell responses in peripheral blood mononuclear cells of 24 lung transplant recipients and 41 healthy controls, to a pool of overlapping peptides from four immediate early antigens as well as a pool of overlapping peptides from four late antigens. We used a denoised ragged pruning algorithm for bias-mitigating clustering of CMV-responsive CD4+ and CD8+ T cells. From these clusters, we are testing for CD4+ and CD8+ T cell phenotypes with significant predictive value for cumulative rejection risk. Such analyses allow us to assess if certain phenotypes of the pre-transplant CMV-specific T cell response could identify patients at high vs. low risk of post-transplant rejection, leading to a more targeted post-transplant intervention.

F231. Assessment of TCR signal strength of antigen-specific memory CD8 T cells in human blood

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Assessment of the quality and the breadth of antigen (Ag)-specific memory T cells in human samples is of paramount importance to elucidate the pathogenesis and to develop new treatments in various diseases. TCR signal strength, mainly controlled by TCR affinity, affects many fundamental aspects of T cell biology, however no current assays for detection of Ag-specific $CD8^+$T cells can assess their TCR signal strength in human samples. Here we provide evidence that interferon regulatory factor 4 (IRF4), a transcription factor rapidly upregulated in correlation with TCR signal strength, permits the assessment of the TCR signal strength of Ag-specific $CD8^+$T cells in human PBMCs. Co-expression of IRF4 and CD137 sensitively detected peptide-specific $CD8^+$T cells with extremely low background in PBMCs stimulated for 18 h with MHC class I peptides. Our assay revealed that human memory $CD8^+$T cells with high-affinity TCR display an intrinsic property to express highly CD25. Furthermore, HIV-specific $CD8^+$T cells in chronic HIV^+subjects were found to display primarily low-affinity TCR with low CD25 expression capacity. Impairment in the functions of HIV-specific $CD8^+$T cells might beassociated with their suboptimal TCR signals as well as impaired responsiveness to IL-2.

F243. Development of a 20-color flow cytometric assay to identify AML patient abnormal blasts and quantify levels of surface and nuclear protein biomarkers

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Acute myeloid leukemia (AML) occurs when myeloid stem cells become immature myeloblasts in bone marrow and is the most common acute form of leukemia (~20,000 cases/year) in the United States. In 2009, the EuroFlow consortium developed a standardized 7 staining tube, flow cytometry (FACS) panel to classify French-American-British (FAB) AML subtypes based on 8-color capable clinical FACS equipment commonly available at that time. Recent developments in FACS instrumentation and fluorochrome chemistries now allow detection of >30 fluorescent markers from a single specimen staining. We are developing and evaluating the performance of a 20-color, 2 staining-tube, FACS assay capable of identifying and quantifying AML patient abnormal blasts present in bone marrow aspirate of AML patients, while simultaneously quantifying levels of either one surface associated or one nuclear associated protein biomarker, using phycoerythrin and allophycocyanin conjugated antibodies in combination with quantitative bead standards. We have used frozen aliquots of bone marrow mononuclear cells (BM-MNCs) from all FAB (M0-M7) AML subtypes to identify abnormal blasts from normal immune cells, and we have evaluated the use of a rapid freezing matrix, SmartTube™. If these studies determine the AML specific biomarker assays are sufficiently robust, this approach would enable easy adoption in the clinical and clinical development setting, and facilitate sample processing/analysis at clinical FACS laboratories trained in AML phenotyping and possessing appropriate FACS equipment.

F244. An Optimized, Automated Workflow for Single-Cell T Cell Receptor Sequencing (scTCRseq)

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T cell receptor (TCR) is a heterodimeric surface protein consisting of alpha-beta or gamma-delta chains. The somatic VDJ recombination in the complementarity determining regions (CDR) of these chains results in specific antigen recognition by T cells. This generates more than 10^{18} possible combinations. This dynamic clonal expansion of T cells after antigen recognition unfolds the unique story of every disease an individual encounters.

Single cell TCR sequencing (scTCR) is used to identify TCR clonotypes, T cell diversity along with targeted gene expression profiles at different stages of a disease. Our lab has optimized a high throughput workflow for scTCR sequencing using automated workstations to systematically study antigen specific T cells. This workflow includes sorting of CD69+CD154+ T cells after a short-term stimulation with a specific antigen into 96 well barcoded plates. Liquid handlers are used to process 4-6 plates simultaneously for library preparation. To increase assay sensitivity, alpha, beta and phenotypic gene amplification is performed separately - includes three rounds of nested PCR amplification along with custom barcoding. Individual libraries are sequenced together using Illumina NGS platform and the data generated is parsed using a custom, in-house pipeline towards analysis. Data from the pipeline is uploaded to our web-based application for quality control, visualization, data management and sharing from our dedicated database. The analysis can be summarized in heat maps or dendrograms to show clonal relationships.

This optimized, automated high-throughput workflow has consistently increased data quality, reduced cost and improved turn-around time for processing large number of samples.

F261. Ribonuclease 7 Level Amongs Pregnant Women With Urinary Tract Infection Attending Tertiary Healthcare Center in Nigeria

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Ribonuclease 7 (RNase 7) is a peptide secreted by intercalated cells of the kidney, it is a potent broad-spectrum antimicrobial agent that can wading off uropathogenic organisms This study was carried out to determine the RNase 7 level among pregnant women with urinary tract infection(UTI) attending antenatal care at tertiary healthcare facility in Nigeria. Mid-stream urine, 150 samples, was collected and cultured for the presence of bacterial pathogens, using >105 colony forming unit as significant level of bacteriuria. 100 samples (40 from those with significant bacteriuria and 60 from non-significant bacteriuria) were randomly selected from this for RNase 7 analysis. Urinary secretion of RNase7 was not statistically significant when compared those with significant bacteriuria and non-bacteriuria ($p > 0.05$). Gestation age and age the pregnant women do not affects RNase 7 urine secretion. There was an association between bacterial infection and pus cells ($p=0.001$). Parity do not affect UTI ($p > 0.05$) however secretion of RNase 7 were statistically significant at third and fifth parity ($p< 0.05$). Bacterial isolates include: Escherichia coli 17(42%), Staphylococcus aureus 10(25%), Staphylococcus saprophyticus 6(15%), Proteus spp 4(10%) and Pseudomonas aeruginosa 3(7.5%). Significant association were obtained between type of bacteria and urine secretion of RNase 7($p< 0.05$), RNase 7 secretion were higher among women with Pseudomonas aeruginosa while Staphylococcus saprophyticus showed the least secretion. Secretion of RNase 7 during UTI among pregnant women was low when compared with those women without infection, thus there is possibility of other factors affecting the secretion hence predisposing pregnant women to UTI.

F301. Simultaneous identification of autoantigens and infectious exposures using randomized peptide display libraries

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Autoimmunity is driven by a complex mixture of genetic predisposition and immune exposures that can lead to the emergence of diverse autoantibodies in patients. While the breadth of genetic involvement in autoimmune disease has been thoroughly studied, immune responses have not been well-characterized with high-throughput tools. Targeted tests for particular autoantigens or infections might be easily run, but identifying autoantigens at a proteome scale (protein and peptide arrays) or assaying many infections (running many tests) is time consuming and expensive. We leverage Serum Epitope Repertoire Analysis (SERA) [an assay based on a random bacterial display peptide library coupled with NGS] and Protein-based Immunome Wide Analysis Study (PIWAS) [a computational tool for the identification of proteome-based signals] to monitor immune status of patients with autoimmune

diseases. We identify both established and novel autoantigens in populations of patients with autoimmune diseases, including systemic lupus erythematosus, Sjogren's syndrome, and myasthenia gravis. We provide peptide-level resolution for each antigen, enabling the identification of dominant epitopes. In one of these PIWAS analyses, we compare 60 SLE samples to 1,157 apparently healthy controls and identify strong signals against Smith family members with conserved peptide epitopes. We confirm this protein and peptide signal using a cohort of 34 predicate anti-Smith patients. Simultaneously, our set of 30+ immunogenic exposure panels for various viral, bacterial, fungal, and parasitic diseases provides insight into exposures that might be associated with the onset of autoimmunity. By leveraging randomized peptide libraries, we identify autoantigens and describe infection history using a single assay.

F303. Expansion of a 30-Marker Standardized Immune Monitoring Assay for CyTOF to Enable Identification of MDSC and Checkpoint Markers

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Mass cytometry, which utilizes CyTOF® technology, is a single-cell analysis platform that uses metal-tagged antibodies. CyTOF can resolve more than 50 parameters in a single panel without the need for signal compensation or deconvolution, making it an ideal solution for fast, routine enumeration of immune cells. The Maxpar® Direct™ Immune Profiling Assay™ is an optimized 30-marker panel contained in a dry single-tube format for human whole blood or PBMC staining, and samples are acquired on the Helios™ mass cytometer. Maxpar Pathsetter™ is an automated software that reports cell counts, percentage calculations, and staining intensity for 37 immune cell populations identified by the assay. The panel can be tailored by adding markers to open channels, and the Maxpar Pathsetter model can then be customized to measure expression markers on existing classified populations or identify additional immune cell subsets.

We present data where the Maxpar Direct Immune Profiling Assay serves as a core immunophenotyping panel and additional markers are added to create a nearly 50-marker panel. The added markers are used to identify MSDC, further classify existing cell populations, and measure immuno-oncology-related markers including OX40, Tim-3, Fas, PD-1, PD-L1, ICOS, and TIGIT. We demonstrate how the Maxpar Pathsetter model is modified to incorporate the added markers.

The ability to expand the Maxpar Direct Immune Profiling Assay and customize the Maxpar Pathsetter software model shows the power and flexibility of the system to enable fast yet customized deep immune profiling.

F358. Impact of a new ALC-based dose of Thymoglobulin® (ATG) on the early immune reconstitution after αβhaplo-HSCT in pediatric patients with hematological malignancies.

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Anti-thymocyte globulin (ATG) is used before αβ T-cell/CD19 B-cell depleted haploidentical allogeneic hematopoietic stem cell transplantation (αβhaplo-HSCT) to prevent graft rejection and graft-versus-host disease. We compared two different ATG doses in 27 pediatric patients with hematological malignancies undergoing αβhaplo-HSCT. In Cohort 1 patients received a fixed dose of 3.75 mg/Kg, while in Cohort 2 they received a dose calculated using a novel algorithm based on absolute lymphocyte count (ALC) and body weight. We defined CD4 immune reconstitution (IR) as $CD3^+CD4^+$ T cells > 50 cells/ul twice within 100 days after HSCT. Overall, 70% of patients achieved CD4 IR: 85.7% in Cohort 1 and 54 % in Cohort 2. However, the ALC-based regimen resulted in the more pronounced reduction of donor-derived memory T cells. In Cohort 2, αβ T cells were significantly lower at Day 30 and Day 90 (P=0.0003). At Day 90, CD4 and CD8 were significantly depressed (P=0.01 and P=0.056, respectively). In both subpopulations, the memory compartment was the most reduced. Our results suggest that the selective depletion of the memory compartment in CD4/CD8 is due to a priming effect of ATG on the few αβ T cells residual in the graft, whereas the equivalent reconstitution of naive T cells in both Cohorts is likely because of no impact of ATG on the thymus-dependent IR. *In vivo* studies of Thymoglobulin® pharmacokinetics in αβhaplo-HSCT recipients and a comparison with the use of Grafalon® are required to shed more light on this crucial topic.

TH6. Data Integration and Comparative Analytics using the Extended Polydimensional Immunome Characterization (EPIC) platform

Salvatore Albani[1], Joo Guan Yeo[2], Martin Wasser[2], Pavanish Kumar[1], Su Li Poh[2], Fauziah Ally[2], Thaschawee Arkachaisri[3], Amanda Jin Mei Lim[2], Jing Yao Leong[2], Anis Larbi[4], Tze Pin Ng[5], Liyun Lai[2], Kee Thai Yeo[6], Elene Seck Choon Lee[3], Camillus Chua[2], Bhairav Paleja[2], Angela Yun June Tan[3], Shu Ying Lee[3] and Florent Ginhoux[7]

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The lack of a cogent approach for cross-datasets comparison and harmonization to capitalize on the growing mass cytometry data in online repositories for mining is a critical unmet need. Unlocking this capability will catalyze scientific discovery by increasing sample size to achieve greater statistical power for more meaningful analysis.

We interrogated >200 healthy peripheral blood mononuclear cell samples (cord blood to 95 years old) with 2 mass cytometry panels encompassing 63 phenotypic and functional markers. Quality control and batch normalization was done to create a standardized, high-dimensional Immunome database for comparison using a web-enabled, R-shiny EPIC platform.

We downloaded healthy data (18 to 90 years old, n=98) from 2 Immport studies (SDY113, SDY420) for uploading to EPIC where automated downsampling, normalizing and FlowSOM (Flow analysis with self-organizing maps) clustering with the database to 20 clusters (k) using 6 common markers (CD3, CD4, CD8, CD14, CD19, CD56) were done. Overlap with comparable ages in EPIC was observed on correspondence analysis (CA) plot where proximity denotes similarity in immune compositions. CA plot of SDY113 (n=58) with diverse racial groups and EPIC (k=30, 8 markers with addition: CD27, IgD) shows similarity of Asians with EPIC.

We verified the unsupervised frequencies (8 defined subsets) and correlated them with bi-variate gated frequencies (Pearson's correlation coefficient: 0.8056 to 0.9921, $p < 0.0001$). We have successfully harmonized disparate datasets after comparability assessment using the EPIC platform. Greater experimental standardization and markers overlap will enhance datasets merging for re-discovery.

TH7. Extended Polydimensional Immunome Characterization (EPIC) Platform as a Tool for the Multi-dimensional Characterization of Immune Aging

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We created a high dimensionality healthy human Immunome atlas by interrogating the peripheral blood mononuclear cells (PBMC) of >200 healthy subjects (cord blood to adult) with 63 unique mechanistic and phenotypic markers per cell by mass cytometry (CyTOF). This database is built with an open source, web-based bioinformatics toolkit, enabling its mining and datasets uploading for comparison.

CyTOF data from 37 healthy elderly (>60 years old) were uploaded onto EPIC Discovery tool where down-sampling, normalizing and FlowSOM clustering were done. Visualization tools include cluster frequency boxplots, correspondence analysis (CA) plot and markers expression heat-map. CA plot depicts the global differences in immune cells composition between subjects with proximity between

points (subjects) denoting similarity. Kruskal-Wallis test was done to identify age groups differences.

Increasing distances on CA plot with age were observed with the elderly being farthest from the newborns. Notably, we observed significant changes in naive CD4+ IL8+ T cells ($p< 1\times10^{-20}$), memory CD4+ IL17A+ T cells ($p< 1\times10^{-20}$) and type 2 innate lymphoid cells (ILC2) (Lin- CD7+ CD25+ CD127+ CD161+, $p< 1\times10^{-17}$) with increasing age. The naive CD4+ IL8+ T cells (median: 0.68%, interquartile range: 0.415 to 1.055% of CD45+ PBMC) and ILC2 (0.09%, 0.065 to 0.12%) were lowest and memory CD4+ IL17A+ T cells (0.58%, 0.41 to 0.905%) highest in the elderly.

With EPIC, we have created an online tool enabling data uploading for comparison to a healthy database, allowing the holistic characterization of immunological changes in different clinical scenarios.

TH22. Machine learning to automate cellular biomarker identification for clinical diagnosis from cytometry data

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Flow and mass cytometry are commonly used for monitoring the cellular complexity of the immune system to understand responses to natural (e.g. infections, cancer) and designed (e.g. vaccines, therapeutics) perturbations and to discover cell-based biomarkers of disease. Over the last decade, the bioinformatics community has developed a series of computational methods to analyze the high dimensional data generated by these cytometry platforms. Here we report the development of a computational pipeline that includes unsupervised clustering, supervised machine learning and non-linear embedding dimensionality reduction to automatically identify cell-based diagnostic biomarkers for classifying samples/patients into discrete phenotypic categories. Performance of this diagnostic pipeline has been assessed using data collected by the UCSD Center for Advanced Laboratory Medicine for the clinical diagnosis of chronic lymphocytic leukemia (CLL). The pipeline showed excellent classification accuracy in comparison with expert manual analysis and automatically identified the diagnostic cell populations with interpretable gating locations optimized by the machine learning component. The pipeline was also robust to recognize atypical CLL phenotypic heterogeneity, due to the non-linear embedding component. Although the pipeline was developed for the clinical diagnosis of CLL, the methodology developed can be easily applied to any circumstance in which sample/patient classification is the objective.

TH60. Defining Cell Fitness Criteria to Increase the Reliability of Biomarker Assays which use Cryopreserved Peripheral Blood Mononuclear Cells

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Peripheral blood-based assays are often used to develop biomarkers for the diagnosis and therapeutic response tracking of a wide range of diseases. However, biomarker research is notoriously hampered by reproducibility problems which can be exacerbated by the use of cryopreserved peripheral blood mononuclear cells (PBMCs). In particular, poor cell fitness can contribute to erroneous results. Despite the importance of cell fitness, (often assessed by viability), there are currently few evidence-based standards for minimal cell viability for many commonly used assays. In this study, we used an 'induced fail' approach to examine the impact of cell viability on four flow-cytometry based assays. Two assays involved direct phenotyping (leukocyte and T_{helper} cell profiling) and two involved measuring the response to inflammatory signals (IL-6 or LPS stimulation of pSTAT3 or cytokines, respectively). We found that cell permeability-based viability stains at the time of thawing are not accurate indicators of cell fitness and suggest an alternative viability indicator based on metabolic activity and exclusion of cells in early apoptosis (live, apoptosis-negative or LAN). We also found that the impact of cell damage on PBMC sub-populations was dependent on both the type of damage and the type of cell. Our data shows that assay results from samples with LAN < 60% were more highly influenced by cell fitness than individual subject characteristics, making this our recommended viability cut-off for these types of assays. Incorporation of minimal cell viability criteria into standardization protocols is essential to facilitate the discovery and reproducibility of immunological biomarkers.

TH77. Immunomonitoring for Response and Adverse Events in Hepatocellular Carcinoma (HCC) Patients Undergoing Immunotherapy

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Immune checkpoint blockade (ICB) represents one of the frontline therapeutic interventions in cancer immunotherapy that aims to re-activate the cytotoxic potential of the patient's CD8 T cells against the tumour. However, its **response rate** in various solid tumours is only modest (20-30%), and some patients also develop **immune-related adverse events** such as rashes and colitis. It is hence critical to understand the molecular underpinnings of these immune responses in order to predict and tailor treatment strategies for improved clinical outcome.

Our team profiled the peripheral blood mononuclear cells (PBMCs) isolated at various time points from Hepatocellular Carcinoma (HCC) patients who had undergone immunotherapy using Cytometry by Time of Flight (**CyTOF**). We aim to capture immune responses and modifications related to response and adverse effects upon ICB and to correlate the immune profiles obtained from the peripheral blood with that in the local anti-tumour response.

Our immunoprofiling data demonstrated an increase in myeloid antigen-presenting cells (APCs) in PBMCs of HCC patients responding to immunotherapy with either sustained partial response or stable disease beyond 6 months upon ICB. An increase in central memory CD4 T cells was also observed in these patients. Further validation on the immune cell subsets is currently being done.

Equiped with high and multi-dimensional immunoprofiling tools, we aim to identify **biomarkers** and **understand the mechanisms of action** behind clinical response or adverse events following ICB in HCC patients.

TH112. Beyond the margins: novel fluorochrome development enables 34-color high-content flow cytometry

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Flow cytometry is an essential technology to identify and quantify cell populations. In this contribution, we examine the development of new fluorochromes that could enhance flow cytometry's capability. The latest spectral flow cytometers have 3 excitation lasers (405nm, 488nm, and 640nm) and incorporate the Avalanche Diodes Photodetector technology, which demonstrates significant improvement in sensitivity for the fluorescent emission signal longer than 800nm. However, there is no commercially available fluorochrome which could be excited by the above lasers and has peak emission beyond 800nm. To address the gap in technology, we engineered 6 new fluorochromes: PE-750, PE-810, PE-830 for blue laser and APC-750, APC-810, APC-830 for red laser. These were created by covalently linking a protein donor dye with an organic small molecule acceptor dye. Then, the fluorochromes were conjugated with antibodies using Click chemistry, which is a simple, robust reaction with high-yield and high-reaction sensitivity. Also, we demonstrated long-term stability at -20^{o}C with protein stabilizing cocktails. Most importantly, in order to show the utility of these novel fluorochromes, we created a 34-color flow cytometry panel to measure broad human immune function with high sensitivity on a 3-laser Aurora. The additional UV laser upgrade will allow head-to-head comparison with multicolor panel optimized on BD FACS Symphony and ultimately increase the capacity to more than 40 color.

In conclusion, this high-content flow cytometry panel will improve analysis and diagnosis in Immuno-oncology and facilitate innovation in biomarker discovery.

TH129. The role of HLA-DR expression on monocytes and Sepsis Index as predictive sepsis biomarkers

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From the immunological point of view, sepsis is characterized by an unbalanced host response to an infection. The identification of biomarkers able to identify those patients prone to develop sepsis is essential to reduce mortality and morbidity rates. In this context, the surface expression of HLA-DR molecules on monocytes (MHLA-DR) and of CD64 molecules on neutrophils (NCD64) have shown to be useful parameters in different settings. The Sepsis Index, defined as the ratio between NCD64 and MHLA-DR allows us to assess the presence of sepsis. In this study, we analyzed the relationship between immunological parameters (MHLA-DR, NCD64 and Sepsis Index) and the probability to progress to sepsis in patients admitted to Intensive Care Unit (ICU). A prospective longitudinal study of 77 no-infected patients admitted to ICU due to severe neurological injury was performed. The MHLA-DR and NCD64 were analyzed in whole blood samples at admission, +3, +6, +9, +12 and +15 days after admission, using a standardized flow cytometry protocol. During the follow-up, 71% of patients became septic. Analysing retrospectively, we found that, before sepsis diagnosis, septic patients showed a lower MHLA-DR rate and a higher Sepsis Index than those patients that did not develop sepsis ($p = 0.001$). We conclude that the immune-monitoring of MHLA-DR and Sepsis Index may help to identify those patients with higher susceptibility to develop sepsis at the ICU, and facilitate their management.

TH191. Optimizing TCR Repertoire Profiling Technologies for Precise Human Immune Monitoring

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T cell receptors (TCR) have been widely studied to monitor the dynamics of T cells in terms of clonality, convergence, and diversity in human disorders, using the V segments (CDR3) sequencing of αβT and/or γδT chains (TCRseq). However, the techniques for the detection are still facing challenges due to technical bias, which may significantly compromise the accuracy of TCR profiling.

We here compared three major methods, nested RT-PCR (custom), 5'RACE (v1), and 5'RACE with UMI (v2), on bulk TCRseq. Our results indicated that all three methods worked consistently well in terms of library preparation and sequencing quality. The custom method had the simplest procedure with highest TCR calls and minimum cost but needs a longer 2x250 sequencing run for joining paired sequences to assemble complete CDR3 on a customized VDJ analysis pipeline. The v1 method required the longest 2x300 sequencing run to cover full length of CDR3 v gene. Lower TCR calls and fewer clonal types were identified. The v2 method dramatically enhanced specificity and sensitivity. Moreover the v2 libraries can be sequenced in a shorter length 2x151 run, compatible with higher throughput platforms. It should be the most cost-effective option for large scale TCRseq. Both v1 and v2 sequences can be easily analyzed using commercially available MiXCR software.

In conclusion, the tested methods had user friendly workflows, high reproducibility, sensitivity & specificity. The major clones with similar CDR3 length distributions were called successfully. An appropriate method should be selected depending on experimental design and feasibility.

Immunodeficiency (primary or acquired)

TH132. Development of a Clinically Validated Test for DOCK8 Protein Expression

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Dedicator of Cytokinesis 8 (DOCK8) is an atypical guanine exchange factor that plays a role in regulating actin polymerization and cytoskeletal rearrangement. DOCK8 is important in both innate and adaptive immunity by contributing to cellular migration, cytotoxicity, antibody production and immunological memory.

DOCK8 deficiency is an autosomal recessive disorder that typically presents in childhood. It is characterized by atopic disease, cutaneous viral infection, and recurrent sinopulmonary infections. DOCK8 deficiency is diagnosed based on clinical phenotype, immunologic findings and molecular analysis. Assessment of DOCK8 expression on immune cells is an important aspect of these studies. Currently no US clinical reference laboratory provides this assessment.

To address this diagnostic need, we have developed a whole blood immunophenotyping assay to evaluate DOCK8 expression on T-cells (CD45+CD14^{neg}CD3+), B-cells (CD45+CD14^{neg}CD3^{neg}CD19+), NK-cells (CD45+CD14^{neg}CD3^{neg}CD56+) and monocytes (CD45+CD14+). To establish a reference range, the assay was performed on blood obtained from 158 unique normal donors, including 118 pediatric donors, 3 months to 17 years of age (49.2% males, 50.8% females), and 40 adults between 25-76 years (55% males, 45% females). The reference range was determined by calculating the upper 5th-percentile of the data using quantile regression.

The test's clinical utility was confirmed on 2 patients with compound heterozygous mutations in DOCK8; and their parents, each bearing 1 mutation. The patients had no expression of DOCK8 on monocytes, B-cells or NK-cells but had DOCK8 expression on a fraction of T-cells, consistent with somatic reversion in their memory T-cells. The parents had normal expression of DOCK8 on all cell types.

TH154. Molecular Characterization of a Hong Kong Chinese Boy with Wiskott-Aldrich Syndrome

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Wiskott-Aldrich Syndrome (WAS) is a rare X-linked recessive immunodeficiency syndrome characterized by thrombocytopenia, eczema, recurrent infections, increase in risk of developing autoimmunity and haematological malignancies. It is caused by mutations in the Wiskott-Aldrich Syndrome protein (*WASP*) gene. We investigated a Hong Kong Chinese 7-month boy who presented with neonatal thrombocytopenia, eczema and autoimmunity. A novel mutation which was a single T insertion in exon 4 of *WASP* gene by sequence analysis was found. This T insertion results in a frameshift mutation with a substitution of a lysine to an asparagine at amino acid 144 followed by a premature stop codon (p.Lys144AsnfsTer25) and resulted in a truncated protein. WAS should be one of the differential diagnosis in a male child with the presentation of early onset of thrombocytopenia, particularly with eczema and autoimmunity. Early diagnosis might reduce related complications and increase life expectancy. Furthermore, mutation analysis is importance for the diagnosis of WAS and also for carrier detection and prenatal diagnosis by expanding the spectrum of *WASP* mutations.

TH195. Multiplex Proteomics can Identifiy Immune Dysregulation Phenotype from Infection Only n Common Variable Immunodeficiency.

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Patients with common variable immunodeficiency (CVID) can develop inflammatory complications such as autoimmunity, lymphoproliferation, enteritis and malignancy, which causes significant morbidity and mortality. Better understanding of the pathophysiology of these complications is required to improve screening and treatment for these patients. We identified and validated serum biomarkers for immune dysregulation in CVID using Protein Extension Assay panels consisting of 180 markers in two independent multicenter CVID cohorts. In the discovery cohort consisting of CVID with immune dysregulation (CVIDid, n=15), CVID with infection only (CVIDio, n=15) and healthy controls (HC, n=15), unsupervised clustering revealed a classification similar to the three clinical phenotypes studied. In a supervised analysis, IL-10, IL-17A, CXCL10, CCL19, CCL20, CXCL9, LAG3, FCRL6 and KLRD1 were upregulated in CVIDid compared to CVIDio (FDR corrected p value < 0.05, fold change > 2). In the validation cohort, IL-10, LAG3, TNFRSF9, CCL19 and KLRD1 were reproducibly upregulated in CVIDid (n=28) vs CVIDio (n=28) ($p < 0.05$). An elastic net algorithm trained on the discovery cohort and tested on the validation cohort reached sensitivity of 75% and specificity of 68% in classifying CVIDid from CVIDio, using IL-12RB1, IL-10, BTN3A2, LILRB4, MILR1, IL-17A, LAG3 and CXCL11 as variables. Pathway analysis of differentially expressed proteins highlighted prominent Th17 activation in patients with CVIDid.

TH378. Gene Correction of a Bare Lymphocyte Syndrome Mutation Restores HLA class II Expression

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Targeted genome editing allows for seamless correction of mutations in living cells. In this study, we explored a gene repair strategy to correct a donor splice site mutation in exon 13 of CIITA, the transcriptional master regulator required for the concerted expression of Human Leukocyte Antigen (HLA) class II genes. Patients with genetic defects in *CIITA* are severely immunocompromised due to defective HLA class II expression and are collectively referred to as Bare Lymphocyte Syndrome (BLS) type II patients. We successfully reprogrammed an EBV-transformed B lymphoblast cell line from a BLS type II patient into induced pluripotent stem cells (iPSC) harboring the underlying donor splice site mutation. Mutant iPSC could readily be differentiated into macrophages deficient in HLA class II expression, thus recapitulating the patient's phenotype. We employed the CRISPR/Cas9 system to facilitate integration of an oligonucleotide repair template designed to restore the donor splice site in the mutant iPSC. When gene-corrected iPSC were differentiated into macrophages, HLA class II expression was observed upon IFNg treatment, indicating that CIITA function was restored. Indeed, PCR across the donor splice site followed by sequencing confirmed correction of the splicing defect. When applied to CD34+ hematopoietic stem and progenitor cells (HSPC) obtained from BLS type II patients, the gene correction approach described here should rescue HLA class II expression in the hematopoietic system and thus enable autologous cell therapy for patients with this particular point mutation.

TH389. STXBP2 R190C monoallelic mutation associated with hemophagocytic syndrome acts as a dominant negative in vitro.

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Hemophagocytic lymphohistiocytosis (HLH) is a multisystem hyperinflammatory syndrome characterized by persistent macrophages/lymphocytes activation.
Here, we describe a 9-month-old child diagnosed at 2-months of age with cutaneous Langerhans cell histiocytosis (LCH) and treated according LCH-III protocol. The disease progressed to multisystemic form at 7-months of age, the patient was treated with cytarabine/cladribine with partial response. Nevertheless, the patient developed a full HLH picture, (5/8 criteria). The patient underwent HSCT at 16-months of age from a mismatched related donor, but the graft was loss 5-months later concurrent with an uncontrolled adenoviral infection and HLH relapse. Impaired NK-cytotoxicity was observed in both episodes with a recovery between them.
The sequencing of the genes involved in primary HLH only revealed one low-frequency heterozygous variant in *STXBP2* gene (c.568C >T/p.R190C). Familial study showed that this variant was inherited from his mother. We investigated the effect of *STXBP2* monoallelic mutation R190C; COS-7 cell transfection was done with constructs STXBP2-WT, STXBP2-R190C and STX11-WT. Protein expression levels by western blot demonstrated that the mutation STXBP2-R190C showed similar expression compared wt protein. Co-immunoprecipitation experiments demonstrated that STXBP2-R190C maintained the ability to interact with STX-11. To examine the functional effect on the protein, RBL-2H3 cells stably expressing EGFP-STXBP2-R190C showed a significant reduced degranulation compared to EGFP-STXBP2-WT and non-transfected RBL-2H3 cells. These data suggest that STXBP2-R190C acts as a dominant negative in this *in vitro* model.

The functional and molecular characterization of this particular mutation would indicate the recommendation of regular medical follow-up in carrier patients who had developed HLH.

Immunology of the Eye

TH41. Retinal Pigment Epithelial Cells Regulate Antigen Presentation

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Retinal pigment epithelial cells (RPE) release soluble factors that suppress phagosome maturation and phagolysosome activation within antigen presenting cells (APC). This suggests that within the retina there is altered antigen processing and presentation. Therefore, we assayed the effects of the RPE soluble mediators on the ability of APC to stimulate antigen-specific $CD4^+$ T cell proliferation,

differentiation, and cytokine production. Also, we assayed RPE from eyes with autoimmune uveitis. Peritoneal macrophages from naive C57BL/6 mice, were cultured with opsonized-ovalbumin (OVA), and treated with conditioned-media from cultured RPE-eyecups. The RPE-eyecups were dissected from naive and uveitic mouse eyes consisting of the RPE monolayer, underlining choroid and sclera. The eye-cups were incubated in serum-free media for 24 hrs., and cell-free conditioned-media was collected. The irrelevant-antigen control was opsonized-rat serum albumin. The treated APC were washed, and $CD4^+$ T cells from mice immunized to OVA were added. Antigen-stimulation of $CD4^+$ T cell proliferation was suppressed when the APC were treated with conditioned-media from naive RPE-eyecups, and not from uveitic RPE-eyecups. While APC treated with naive RPE-eyecup conditioned-media did not expand $FoxP3^+CD25^+$ Treg cell population, APC treated with the conditioned-media of uveitic RPE-eyecups had a 60% diminished Treg cell population. There was no statistical difference in antigen-stimulated IFN-γ, IL-17, and IL-10 concentrations between T cells stimulated with APC treated with either naive or EAU-RPE eyecup conditioned-media. These results demonstrate that APC are influenced by RPE soluble molecules, and suggest that RPE promote APC maintenance of Treg cells within ocular immune privilege.

TH67. Commensal Bacteria May Contribute to Ocular Manifestations Characteristic of Autoinflammatory Diseases Associated with NLRP3 Gene Mutations

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We reported previously (PMID: 28709803) that ocular surface of healthy wild-type mice is colonized by a commensal, common to mice and humans, *Corynebacterium mastitidis* (*C. mast*). On the murine ocular surface, *C. mast* elicits IL-17 from T cells and promotes local host defense. Based on this we hypothesized that ocular inflammation in Autoinflammatory disease patients with *NLRP3*-associated mutations may be triggered by an overactive response to ocular surface bacteria. We found that, unlike wild-type mice, knock-in mice bearing an *NLRP3* gene mutation found in patients, developed conjunctivitis, conjunctival neutrophilia, and increased IL-1 and IL-17 responses following ocular exposure to *C. mast*. scRNA-seq of murine conjunctival cells confirmed that γδ T cells were a major immune cell population and a source of *Il-17a*, possibly explaining the neutrophilia, and expressed *Ccr2* and *S100a8* transcripts. Importantly, patient PBMCs stimulated with *C. mast* lysate overproduced IL-17A compared to healthy controls, as measured by ELISA. However, in contrast to mice, scRNA-seq of patient conjunctival cells and PBMCs showed upregulation of Th1 and Th17 signaling molecules in αβ, rather than in γδ, T cells. This suggests that response to *C. mast* in patients may be mediated by different immune cells than in mice, and/or that commensals other than C. mast may drive their ocular phenotype. Our study demonstrates that commensals can elicit exaggerated immune responses in humans and mice with *NLRP3*-related autoinflammatory disease. If indeed conjunctivitis in these

patients reflects a response to ocular surface commensals, adjunct antimicrobial therapy could be considered.

TH97. Single-cell RNA sequencing coupled with B-cell-receptor sequencing reveals clonal expansion and differentiation of activated B cells in granulomatous uveitis.

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Background Understanding of granulomatous uveitis was previously limited due to small sample sizes, however single cell RNA sequencing (scRNA-Seq) enables extensive unbiased phenotypic analysis of small volume ocular fluid biopsies and unprecedented insight into pathophysiologic mechanisms.

Methods

To characterize B cells in granulomatous uveitis, we performed scRNA-Seq on aqueous fluid and peripheral blood from 4 patients. To assess whether antigen-driven immune responses may have occurred, we also analyzed unique B cell receptor (BCR) sequences in these cells.

Results

Ocular B cells were comprised of activated B cells (ABCs), and plasmablasts. Ocular ABCs cells expressed high levels of HLA class II molecules and GO pathway analysis confirmed they were enriched in antigen presentation genes. Additionally, ocular ABCs were transcriptionally similar to those identified in rheumatoid arthritis (RA) synovium. Multiple clonal B cell populations were identified, the largest represented 43% percent of B cells from one patient. Clonal B cells with divergent Ig subclasses, along with AICDA (activation-induced cytidine deaminase) expression by ABCs suggests that class-switching also occurred. Finally, clones were present amongst both ABCs and plasmablasts, suggesting that terminal differentiation also occurred within the eye.

Conclusion

Taken together, these data suggest that antigen-driven clonal expansion, class-switching and plasmablast differentiation occur in ABCs in granulomatous uveitis. Ocular ABCs were similar to synovial ABCs, suggesting that similar pathophysiologic mechanisms occur in both target organs. B-cell targeted therapies, such as rituximab which is effective in RA, may therefore be a viable strategy for treating granulomatous uveitis.

TH317. Multigranular Analysis of Single Cells Identifies Drivers of Wet Age-related Macular Degeneration Pathogenesis

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Age-related Macular Degeneration (AMD) is a debilitating and under studied disease with unknown pathogenic cellular drivers. As a multitude of cell types exist in the eye, both abundant and rare, understanding this tissue in depth and at all levels of granularity is required to understand the disease. To address this, we developed a new approach for coarse graining by using a data-driven time-inhomogeneous diffusion process called Diffusion Condensation (DC). Our algorithm learns a "continuous hierarchy" of coarse-grained representations by iteratively contracting the data-points towards a time-varying manifold. This effectively gives a continuous hierarchical picture of cellular subtypes at all levels of granularity. To comprehensively understand the retina in healthy and diseased states, we generated single cell Nuclear-seq (scNUCseq) of postmortem retinas from diseased and healthy donors and applied DC to create a comprehensive hierarchical organization of the cellular subtypes in the retina. At low levels of granularity, DC identified the known 12 cell type structure. At higher levels of granularity, we uncovered a novel subpopulation of hypoxic cones enriched in AMD patients. This subpopulation secretes saturated fatty acids, a known component of the drusen deposits found in AMD. These lipids mediate inflammation and promote pro-inflammatory loop between microglia and astrocytes that was not only self-amplifying but also pro-angiogenic and neurodegenerative, which may be a key driver the disease. Through this novel clustering tool we have found a signaling framework that not only addresses disease pathogenesis but also propose novel therapeutic targets for intervention.

Immunomodulation

F177. Tyr192 is a critical regulator of lymphocyte-specific tyrosine kinase activity in T cells

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Good health critically depends on tight regulation of the tyrosine kinase Lck, which initiates T cell receptor signaling. Two phosphotyrosines control Lck: the activating $pTyr^{394}$ and the inhibitory $pTyr^{505}$. Recently $pTyr^{192}$ in the Lck SH2 domain emerged as a third regulator, either via altered Lck specificity or via abolished Lck interaction with the phosphatase CD45. To further explore how $pTyr^{192}$ affects Lck function, we generated CRISPR/Cas9 targeted knockin mutated Jurkat T cells. Lck with non-phosphorylated SH2 (Y192F) displayed hyperactivity, possibly by promoting Lck Tyr^{394} transphosphorylation. The Lck Y192E mutant mimicking stable $pTyr^{192}$, was inhibited by Tyr^{505} hyperphosphorylation. To overcome this effect, we also mutated Tyr^{505}. The resulting Lck Y192E/Y505F mutant displayed strongly increased levels of $pTyr^{394}$ both in resting and activated T

cells. A fundamental role of pTyr^{192} may thus be to protect phosphorylated Lck from CD45 in resting T cells, thus fine-tuning Lck for rapid activation.

F181. Development of Kidney Targeted IL-2 Mutein To Address Graft Rejection in Kidney Transplantation

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Kidney transplantation is a life-saving treatment for patients with end-stage renal disease. However, transplant patients require life-long administration of immunosuppressive agents to prevent graft rejection, resulting in burdensome side effects and decreased quality of life. One promising approach to combating rejection without general immunosuppression is Treg cell therapy; however, scalable manufacturing of *ex vivo* Tregs presents significant challenges.
We previously developed a proprietary IL-2 mutein that selectively expands Tregs *in vivo,* eliminating the need to isolate and expand patient Tregs *ex vivo*. Here, we report the development of a kidney targeted IL-2 mutein fusion molecule with the goal of expanding Tregs locally in the kidney. We identified a target with high abundance in the kidney and low expression in other tissues. Using phage display technology, we generated multiple unique antibody clones specific for the kidney target protein with a range of affinities and cross-reactivity for human, mouse and *cynomolgus* monkey orthologues. Kidney target specific antibody clones were then converted into bifunctional molecules coupled with the IL-2 mutein and shown to maintain Treg specificity *in vitro*. *In vivo* localization to kidney tubules was confirmed in mouse kidney by IF. No localization was observed to other tissues tested. *In vivo* studies are also underway to evaluate Treg expansion within the kidney.

F223. Immunomodulatory effects of ranitidine in a phase IV trial of healthy individuals

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Histamine receptor 2 (H2R) antagonists, such as Ranitidine, are commonly used in patients with gastric or duodenal ulcers, dyspepsia or gastroesophageal reflux disease. Ranitidine has been associated with anti-tumor effects in patients with multiple myeloma and advanced malignant melanoma. Experimental murine breast cancer models have demonstrated anti-tumor effects of high-dose ranitidine treatment via the reduction of monocytic myeloid-derived suppressor cells (M-MDSC). Although H2R is expressed by multiple human immune cells, little is known about the immunomodulatory effects of ranitidine. A phase IV clinical study was conducted in 29 healthy subjects without a medical requirement for H2R blockade. Subjects received daily oral ranitidine treatment not exceeding 900 mg/day for 6 weeks. Peripheral blood was taken at baseline, after 3 and 6 weeks of treatment and 12 weeks after cessation of treatment for immunophenotyping and assessment of soluble mediators in plasma. Ranitidine treatment decreased the percentage of circulating polymorphonuclear-myeloid derived suppressor cells (PMN-

MDSC), but not M-MDSC cells. Ranitidine did not affect the percentage or the absolute number of monocytes, neutrophils or total NK cell, $CD8^+$, or $CD4^+$ T cell numbers. The treatment caused a substantial decrease in B cell numbers and a modulation of IL-2Ra (CD25) expression on T and NK lymphocytes as well as IL-2 plasma level. Overall, this study demonstrated substantial *in vivo* changes in human peripheral blood immune cells suggesting potential for therapeutic regulation of PMN-MDSC through H2R receptors and highlighting the impact on B cell and T cell modulation. ClinicalTrials.gov ID: NCT03145012. *This work was supported by* CCRSI

F246. Molecular Profiling Reveals Anti-PD-1 Agonist Antibody-Induced Changes to Key Immune Pathways

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Co-inhibitory receptors play a critical role in the regulation of T cell activity and represent attractive targets for the restoration of immune tolerance in autoimmune disease. Activation of PD-1-mediated signaling is a promising therapeutic strategy to reduce T cell activity. We have generated a set of PD-1 agonist antibodies which inhibit T cell function. Here we demonstrate that *in vitro* gene and protein expression molecular profiling can be used to identify pharmacodynamic biomarkers for anti-PD-1 agonists.

PD-1 agonist antibodies were evaluated in a human primary cell-based assay. RNA was extracted and conditioned media was collected. Gene expression changes in proinflammatory cytokines were measured by qRT-PCR and gene expression profiling on over 700 genes using a Nanostring autoimmune profiling panel was also carried out. Proinflammatory cytokine production was measured by ELISA and a set of over 300 proteins was examined in conditioned media samples using O-link proteomic analysis. Gene set enrichment analysis showed a convergence of molecular adaptations across protein and gene measurements in response to PD-1 agonism, revealing the modulation of key immune pathways and offering a viable strategy for understanding mechanisms associated with agonism of inhibitory receptors and providing a path forward for pharmacodynamic biomarker discovery.

F255. TIM-3 Dependent Temporal Regulation of Restimulation-Induced Cell Death in Human CD8+ Effector T Cells

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Effective immunity relies on tightly regulated homeostatic processes to clear pathogens while avoiding overt immunopathology. Restimulation-induced cell death (RICD) is one such process whereby activated T cells that receive subsequent antigenic stimulation reach a critical T cell receptor (TCR) signal threshold and commit to apoptosis, constraining T cell expansion. We hypothesized that the co-inhibitory receptor T Cell Immunoglobulin and Mucin domain-containing 3 (TIM-3) attenuates RICD

sensitivity by modulating proximal TCR signaling. Remarkably, we found that TIM-3 protected early human CD8+ effector T cells from premature RICD during clonal expansion, but potentiated RICD in late-stage effectors. This functional switch correlated with TIM-3-dependent attenuation or enhancement of TCR signaling in early versus late-stage effectors, respectively. TIM-3 required extracellular ligand(s) to promote RICD resistance in early-stage effectors, but boosted RICD independently of ligand interaction as effectors aged. Consistent with these data, we observed that while TIM-3 localized to the plasma membrane in newly activated T cells, it was largely intracellular at later time points. Preliminary data suggests that differences in TIM-3 localization and function hinge upon CEACAM-1, a TIM-3 ligand that we found to be preferentially expressed in early expanding T cells. Collectively, our data illuminate a novel role for TIM-3 in temporal regulation of RICD sensitivity during a CD8+ effector T cell response. Our findings help to clarify conflicting notions of TIM-3 signaling in human T cells, with important ramifications for emerging immunotherapeutic strategies that target TIM-3 as a "checkpoint" for preventing or reversing T cell dysfunction or exhaustion.

F263. Automation and Rapid Preservation Tools Enable FACS Based Signaling Characterization in Abbvie Clinical Trials

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The stratification of disease treatments combined with the increasing specificity of disease therapies under evaluation in clinical trials necessitates an ability to routinely monitor cell signaling effects in biological specimens collected in the trial setting. Low cost, automated, ex-vivo stimulation and preservation of immune cells present in blood is now possible using electronic systems, such as the SmartTube™ for example, and these tools can assist in obtaining critical information regarding optimal dose and dose regimen selection. We have validated assays demonstrating phosphatase inhibition downstream of IFN-a and IL-23 cell signaling, and, phosphatase enhancement downstream of IFN-a and IL-6 cell signaling in whole blood using automation. Abbvie's adoption of these tools in clinical trials makes it possible to translate into the clinical trial setting nearly identical assays to those that may have been used by an Abbvie Discovery laboratory scientist when characterizing the effects of their development stage clinical candidate.

Disclosure statement: All authors are employees of AbbVie and may or may not own AbbVie stock. AbbVie sponsored and funded the study; contributed to the design; participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final publication.

F284. Salt-sensing by Serum/Glucocorticoid-Regulated Kinase 1 (SGK1) promotes Th17-like functional adaptation of Foxp3+ regulatory T cells

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Foxp3$^+$ Regulatory T cell (Treg) cells are central mediators of self-tolerance and peripheral immune homeostasis. Treg cells must integrate diverse environmental cues to modulate their function for optimal immune regulation, particularly at sites of inflammation. Translation regulation represents a favorable mechanism for the control of gene expression, and for Treg cell environmental-sensing and functional adaptation *in situ*. Here, we carried out an unbiased screen of Treg cell translatome and identified that serum/glucocorticoid-regulated kinase (SGK)1, a known salt-sensor in T cells, as being preferentially translated in activated Treg cells. We show that high salt drives thymic Treg cells to co-express Th17 master transcription factor RORgt and adopt a Th17-like phenotype all-the-while maintaining suppressive function. High salt also enhances the generation of T_H17-like, suppressive induced Treg cells. The effect of high salt on promoting Th17-like Treg cell adaptation is abrogated with either SGK1 inhibition or NKCC1-sodium transporter inhibitor furosemide suggesting a NKCC1-SGK1-dependent mechanism. High salt-mediated Th17-like differentiation of Treg cells was evident in mice fed with high salt diet or injected with high salt pre-conditioned T cells, conditions that associated with increased Th17-driven inflammation in gut and kidney tissues. Overall, our *in vitro* and *in vivo* data show that high salt-promoted RORγt$^+$ Treg cells represent functional specialized subsets of Treg cells that are adapted for regulating Th17-driven inflammation. SGK1 pathway enables Treg cells to integrate environmental signals and adapt their effector function. By understanding these environmental-sensing mechanisms, we envision targeted approaches to fine-tune Treg cell function for better control of inflammation.

F288. Human Mesenchymal Stromal Cells Enhance the Stability of TGF-beta Derived Inducible Tregs During Ex Vivo Expansion

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Inducible regulatory T cells (iTreg) are a promising therapeutic cell source that exhibit Foxp3 expression and suppressive functions similar to natural regulatory T cells. Nonetheless, their clinical potential is limited by the instability of Foxp3 expression and T cell exhaustion that occurs during *ex vivo* expansion. We postulated that mesenchymal stromal cells (MSCs) could enhance the function and stability of iTregs due to their diverse immunomodulatory properties. In this study, we report that human bone marrow mesenchymal stromal cells (BM-MSC) enhanced the stability of Foxp3 expression and augmented Foxp3$^+$ iTreg expansion. In addition, iTreg suppressive function remains more potent during long term culture for 21 days on an MSC co-culture compared to media culture. BM-MSC expanded iTreg exhibit higher surface CD25, CTLA-4, and ICOS expression. Furthermore, we demonstrate a critical role for cell-cell contacts, as Foxp3 expression was not enhanced by BM-MSC conditioned media or in a trans-well culture system. Intriguingly, optical sectioning microscopy and flow cytometry revealed that BM-MSC support iTreg function via direct contact-dependent mitochondrial (mt) transfer.

BM-MSC mt transfer is driven by mitochondrial metabolic function (CD39/CD73 signaling) in proliferating iTreg and promotes iTreg expression of Foxp3 stabilizing factors BACH2 and SENP3. These results demonstrate for the first time cellular and molecular mechanisms that underlie human MSC mt transfer to proliferating cells, thereby enhancing and sustaining their suppressive function in inflammatory conditions *in vitro* and *in vivo*. Notably, our studies indicate a novel role of MSC in maintaining iTreg functions for use as an immunotherapeutic strategy.

F302. Localized Immunomodulation of T cells for Treatment of Autoimmune and Inflammatory Skin Diseases

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Concentration of therapeutic molecules to the tissues that are the sites of autoimmune and inflammatory diseases is a promising approach and may provide improved therapeutic benefit. We have generated bifunctional molecules made up of two moieties. The first binds to tissue-selective proteins, which localizes the bispecific to the tissue of interest. The second is an effector end that targets specific immune pathways to restore homeostasis.

Skin disorders dominated by pathogenic T cells comprise the largest group within the chronic immune-mediated skin diseases. Novel therapies arising from an understanding of T cell biology in the skin could be broadly applicable to pathogenic immune states in other tissues. We describe here the generation and activity of bispecific molecules that target the skin with different immune effectors. Localizing these immune effectors to the skin could drive the resolution of cutaneous inflammation.

We have evaluated immune effectors that inhibit T cell activation and function, immune effectors that selectively expand regulatory T cells, and immune effectors that deplete local ATP. These skin-tethered immune effectors were tested for their target-binding ability and tissue-specific localization. They were further tested in pathway-relevant mouse models where we observed either tether-dependent or -independent effects. We believe, these skin-tethered bifunctional molecules will provide an opportunity for developing new targeted therapies for autoimmune and inflammatory skin diseases.

F384. Serum HBsAg clearance has minimal impact on CD8+ T cell responses in mouse models of HBV infection

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Antibody-mediated clearance of hepatitis B surface antigen (HBsAg) from the circulation of chronically infected patients (i.e. seroconversion) is usually associated with increased HBV-specific T cell responsiveness. However, a causative link between serum HBsAg levels and impairment of intrahepatic $CD8^+$ T cells has not been established. Here we addressed this issue by using HBV replication-competent transgenic mice that are depleted of circulating HBsAg – via either spontaneous seroconversion or by therapeutic monoclonal antibodies – as recipients of HBV-specific $CD8^+$ T cells. Surprisingly, we found that serum HBsAg clearance has only minimal effect on the expansion of HBV-specific naïve $CD8^+$ T cells undergoing intrahepatic priming, it does not alter their propensity to become dysfunctional, nor does it enhance the capacity of IL-2-based immunotherapeutic strategies to increase their antiviral function. In summary, our results reveal that circulating HBsAg clearance does not improve HBV-specific $CD8^+$ T cell responses *in vivo* and may have important implications for the treatment of chronic HBV infection.

F404. Exogenous delivery of indoleamine 2,3-dioxygenase ameliorates Imiquimod-induced psoriasis

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Indoleamine 2,3-dioxygenase (IDO) catalyzes the rate limiting step of tryptophan catabolism via the kynurenine pathway and plays a critical role in promotion of immune tolerance. This enzyme holds promise as a protein therapy candidate but is limited by quick clearance from sites of administration. We have addressed this by fusing IDO to galectin-3 (gal3), a carbohydrate binding lectin, to act as an anchor. Previous work demonstrates anchoring of the fusion up to one week in subcutaneous tissue. Here we demonstrate that IDO-Gal3 reverses disease severity in an Imiquimod (IMQ)-induced psoriasis. Female C57BL/6j mice were purchased from Jackson Laboratory. Mice were sedated, their backs shaved, and treated with depilatory cream. After skin was dried 62.5 mg of 5% IMQ cream was applied daily for 14 consecutive days. A modified version of the psoriasis area and severity index was used to quantify the severity of erythema, scaling, and skin thickening on a scale from 0 to 4. Recombinant IDO-Gal3 was expressed in *E. coli*. Mice received 5 discrete 10 µL injections of IDO-Gal3 (50 µg total) or sterile saline. Clinical scores were compared using Mann-Whitney U Test. Area under the curve was compared using Student's t-test. Cumulative clinical scores of mice treated with IDO-Gal3 were significantly decreased compared to saline treated controls, the same effect was observed across individual criteria. Comparison of area under the curve was also significantly different. These results show that IDO-Gal3 is promising for therapeutic use in psoriasis. Further experimentation is required to determine the mechanism of action.

F416. Piperlongumine Acts as an Immunosuppressant by Exerting Prooxidative Effects in Human T cells Resulting in Diminished TH17 but Enhanced Treg Differentiation

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Piperlongumine (PL), a natural small molecule derived from the Piper longum Linn plant, has received growing interest as a prooxidative drug with promising anticancer properties. Yet, the influence of PL on primary human T cells remained elusive. Knowledge of this is of crucial importance, however, since T cells in particular play a critical role in tumor control. Therefore, we investigated the effects of PL on the survival and function of primary human peripheral blood T cells (PBTs). While PL was not cytotoxic to PBTs, it interfered with several stages of T cell activation as it inhibited T cell/APC immune synapse formation, costimulation-induced upregulation of CD69 and CD25, T cell proliferation and the secretion of proinflammatory cytokines. PL-induced immune suppression was prevented in the presence of thiol-containing antioxidants. In line with this finding, PL increased the levels of intracellular reactive oxygen species and decreased glutathione in PBTs. Diminished intracellular glutathione was accompanied by a decrease in S-glutathionylation on actin suggesting a global alteration of the antioxidant response. Gene expression analysis demonstrated that T_H17-related genes were predominantly inhibited by PL. Consistently, the polarization of primary human naïve $CD4^+$ T cells into T_H17 subsets was significantly diminished while differentiation into T_{reg} cells was substantially increased upon PL treatment. This opposed consequence for T_H17 and T_{reg} cells was again abolished by thiol-containing antioxidants. Taken together, PL may act as a promising agent for therapeutic immunosuppression by exerting prooxidative effects in human T cells resulting in a diminished T_H17 but enhanced T_{reg} cell differentiation.

F441. MTHFD2 as a metabolic checkpoint in regulating effector and regulatory T cell fate and function

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The balance of pro- and anti-inflammatory T cells is critical to maintaining immunologic homeostasis. These T cells subsets are fueled by distinct metabolic programs that provide for their biosynthetic, bioenergetic, and signaling requirements. Differential dependencies on metabolic pathways, therefore, may be exploited to selectively impair or augment T cell subsets as a therapeutic approach to inflammatory diseases. Recently, mitochondrial Methylenetetrahydrofolate Dehydrogenase and Cyclohydrolase (MTHFD2) has garnered attention as a potential target for anticancer therapy. However,

its function in T cells have not yet been studied. We found that in primary T cells, MTHFD2 activity in the one carbon metabolism pathway is essential for purine synthesis and for cell signaling. Specifically, inhibition of MTHFD2 leads to decreased purine levels and accumulation of the intermediate AICAR, a known agonist of AMPK. The mTORC1-AMPK axis is established as a critical regulator of T cell fate and function. Correspondingly, inhibition of MTHFD2 leads to dramatically reduced proliferation in Th17 cells, while promoting aberrant upregulation of FoxP3 and suppressive capacity. In contrast, Treg cells undergo lineage stabilization in low TGFß conditions. We have further shown that these effects are MTHFD2 dependent as they can be rescued by addition of downstream product formate. When applied *in vivo* to Experimental Autoimmune Encephalomyelitis, MTHFD2 inhibition significantly reduced disease severity and pathogenic T cell infiltration of the spinal cord. Thus, MTHFD2 serves as a metabolic checkpoint in the regulation of Th17 and Treg cells, as well as a potential new therapeutic target within the one carbon metabolism pathway.

F451. Cytomegalovirus Disease Modulates Peripheral Blood Lymphocyte Composition in Several Autoimmune Diseases: A Real-World Evidence

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Background: Immunophenotyping plays a pivotal role in diagnosis and management of several diseases. Using this wealth of clinical flow cytometry data stored in electronic health records (EHRs), we have developed a tool for Comparing the 10,000 Immunomes to Datasets from EHRs (CIDEHR). This application was used to elucidate modulatory factors that affect peripheral blood immunophenotypes in patients with autoimmune diseases.

Methods: CIDEHR compares percentage counts of CD4, CD8, CD19, CD56 cells and ratio of CD4/CD8 cells in different disease cohorts to healthy controls. Regression analysis reveals if factors like age, BMI, and any of the 100 diagnosis code present in at least 10% of the cohort being analyzed, affect the immune phenotypes in the disease. In this study, we have analyzed the factors that influence peripheral blood lymphocyte composition in patients with different autoimmune diseases.

Results: 5,454 immunophenotyping measurements from patients with autoimmune diseases like multiple sclerosis, rheumatoid arthritis, psoriasis, autoimmune thyroiditis, discoid lupus erythematous and systemic lupus erythematous were analyzed. Significant deviations in the CD8, CD56 and CD19 cells were noticed in the broad autoimmune disease category. Regression analysis revealed that cytomegalovirus disease (ICD10-B25.9) was significantly associated with decrease in CD4, CD19 cell count and increase in CD56, CD8 cell count in all 6 categories of the autoimmune diseases.

Conclusion: CIDEHR is useful in evaluating flow cytometric differential in real-world disease populations. It provides a real world evidence on immune modulatory effect of the cytomegalovirus virus in chronic diseases as it encodes several immune evasins that alter immune phenotypes.

TH37. Induction of Immune Tolerance to a Microbiome Epitope Induces Clinical Improvement in Rheumatoid Arthritis Via Induction of PD-1 Expressing Regulatory T Cells

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DnaJP1 is a peptide from the HSP dnaJ of Escherichia coli. It shares a homology with HLADRB1 allele associated with RA. DnaJP1 is an proinflammatory T cell epitope in patients with active RA.

In a double-blind, placebo-controlled, pilot phase II clinical trial in RA patients, immunotherapy with dnaJP1 was well-tolerated and resulted in lower disease severity.

To dissect the mechanisms of action, we studied by high dimensionality cytometry PBMC from both the placebo and treatment groups at the treatment beginning and end. Successful epitope-specific immunotherapy reshaped the effector T cell compartment to a more tolerogenic and less pro-inflammatory function and induced PD-1+ Treg. These PD-1+ Treg are more suppressive than PD-1- Treg and their function is mediated by PD-1 expression and TGFβ, as blockade of either resulted in a dampened suppressive function. We sorted PD1+ and PD1- Treg and subjected them to NG RNA Seq. We then interrogated the dataset for the expression of integrins. PD1+Treg have a significantly higher expression than their PD1- counterparts express the integrin combination A4B7, which is paramount identification for immune cells which have transited through the Peyer's patches.

Hence, treatment with dnaJp1 is, to the best of our knowledge, the first ever therapeutic attempt to successfully exploit the interface between the microbiome and the immune system by restoring tolerance to a microbiome peptide, which is the trigger of abnormal inflammatory responses in RA.

TH65. The heparan sulfate mimetic PG545 modulates T cell responses and prevents delayed-type hypersensitivity

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The heparan sulfate mimetic PG545 (pixatimod) is under evaluation as an inhibitor of angiogenesis and metastasis including in human clinical trials. We have examined the effects of PG545 on lymphocyte phenotypes and function. We report that PG545 treatment suppresses effector T cell activation and polarizes T cells away from Th17 and Th1 and towards Foxp3+ regulatory T cell subsets in vitro and in vivo. Mechanistically, PG545 inhibits Erk1/2 signaling, a pathway known to effect both T cell activation

and subset polarization. Interestingly, these effects are also observed in heparanase-deficient T cells, indicating that PG545 has effects that are independent of its role in heparanase inhibition. Consistent with these findings, administration of PG545 in a Th1/Th17-dependent mouse model of a delayed-type hypersensitivity led to reduced footpad inflammation, reduced Th17 memory cells, and an increase in FoxP3+ Treg proliferation. PG545 also promoted Foxp3+ Treg induction by human T cells. Finally, we examined the effects of other heparan sulfate mimetics PI-88 and PG562 on lymphocyte polarization and found that these likewise induced Foxp3+ Treg in vitro but did not reduce Th17 numbers or improve delayed-type hypersensitivity in this model. Together, these data indicate that PG545 is a potent inhibitor of Th1/Th17 effector functions and inducer of FoxP3+ Treg. These findings may inform the adaptation of PG545 for clinical applications including in inflammatory pathologies associated with type IV hypersensitivity responses.

TH110. Role of Exosomes in the Liver Disease Progression

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Exosomes are a type of extracellular vesicles secreted from a variety of cells and carry numerous biological molecules such as nucleic acids, protein, and lipids [1,2]. They are involved in many biological processes including antigen presentation, immune escape and tumor progression [3]. However, their involvement in different disease pathogenesis including liver diseases and immunotherapeutic applications of exosomes are still not clear. In this study, the role of exosomes in liver disease progression in the course of HBV infection, Cirrhosis and HCC were investigated. Furthermore, we also sought to test the anti-tumor activity of ligand-loaded tumor-derived exosomes as an anti-tumor therapeutics in HCC xenografts.

The data demonstrated that isolated exosomes from patients' and healthy donors' peripheral blood plasma have the characteristics that identify them as exosomes. Notably, PD-L1 expression was gradually upregulated on patients' exosomes from HBV carriers to the HCC state. Cytokines that play a major role in inflammation such as, IL-6, IL-8, IL-12, IFNγ, and TNFα showed a slight increase especially in Cirrhosis and HCC group when compared to controls. Besides, plasma IP-10 levels were comparable among the groups. Cirrhosis and HCC groups had the highest IP-10 secretion. Overall, IFNγ and IP-10 levels were elevated in response to patient exosome, in a dose-dependent manner. Similarly, murine splenocyte stimulation showed the gradual increase of IL-6 and IFNγ in a dose-dependent manner regardless of the immunologic background of the mice. Finally, exosome formulations have enhanced the survival of HCC bearing mice by suppressing the tumor growth.

TH144. Identification of Novel T Regulatory Epitopes in Human Serum Albumin (HSA-Tregitopes)

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The discovery of natural regulatory T cell epitopes (Tregitopes) in the Fc and Fab regions of immunoglobulin G (IgG) has enabled our group to leverage natural tolerance to induce antigen-specific tolerance in allergy, autoimmunity, and transplantation. We and others have shown that co-incubation of Tregitopes with antigen *in vitro* leads to reduced proliferation of effector T cells (Teffs) and expansion of antigen specific adaptive Tregs (aTregs). *In vivo* studies demonstrate that treatment with Tregitopes suppresses T cell and antibody responses to co-administered antigens.

We hypothesized that other prevalent serum proteins such as human serum albumin (HSA) might contain Tregitope-like sequences as a mechanism for maintaining peripheral tolerance to ubiquitous proteins. Using our computational analysis tools, we identified multiple candidate HSA-Tregitopes that are promiscuous HLA DR binders and have highly conserved TCR facing residues (*in silico* signature of Tregitopes). Additionally, we have demonstrated that several of these peptides suppress recall response to Tetanus Toxoid (TT) in an *ex vivo* bystander assay using human PBMCs**,** similar to IgG-derived Tregitopes. Inhibition of CD4+ Teff activation and increased Treg/Teff ratio may result from the activation of $CD4^{+}CD25^{+}FoxP3^{+}$ natural Tregs (nTregs) and/or the conversion of TT-specific Teffs to aTregs.

The identification of HSA-derived Tregitopes has implications for use of albumin as a delivery vehicle in drug development. HSA-Tregitopes could be used to restore peripheral tolerance and address the underlying cause of many autoimmune disorders. Its ability to induce tolerance to its own cargo may eventually be added to the already extensive list of albumin functions.

TH160. Can Tolerogenic Food Mitigate Allergy Induction?

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INTRODUCTION: Evaluate the kinetics of oral tolerance or allergy induction to new proteins in the acute phase of intestinal inflammation. METHODS/RESULTS: Adult male C57BL/6 were divided into 10 groups and received two sub epithelial doses of 100mg of peanut protein extract, within 21 days with adjuvant addition in first and then received peanuts for 40 days (oral challenge-OC) for intestinal inflammation induction, except negative control (NC), which continued with commercial chow. Crystallized ovalbumin (OVA) diluted 1:5 v/v in distilled water, depleted by gavage 6h-before, -after and concomitant the onset of the OC was used. Positive control (PC) and NC received saline per gavage. All animals were given two intra-epithelial doses of 100µg-OVA, with a 21-day interval, the first with adjuvant followed by new-OC (OVA-chow) until experiment ending. We evaluated: body weight, food intake, IgG (total) anti-peanut and OVA titers, duodenum and jejunum histomorphometry and T and B lymphocytes profile of mesenteric lymph nodes. No changes in diet intake were observed, except in new-OC. Anti-OVA total IgG antibody titers showed significant increase in concomitant group. Flow cytometry revealed significant decrease in CD4+CD25+Foxp3+ lymphocytes and significant increase in CD8+ T lymphocytes in concomitant group. Histomorphometry, Concomitant and 6h-After groups were classified as Infiltrative and Partial Destruction stages hybrids. 6h-Before was classified as Infiltrative, while PC was classified as Partial Destruction stage. NC was classified as Normal. CONCLUSION:

New protein introduced concomitant to allergenic protein might develop multiple allergies. New food administration in a physiological context of the gut mucosa generates tolerance.

TH315. Inhibition of human CD38 ecto-hydrolase activity with a biepitopic first-in-class fully human mAb that lacks effector function prevents acute GvHD in immune humanized NSG mice

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CD38 is a multifunctional ectoenzyme expressed predominantly on immune cells. Increase of CD38 expression results in decline of intracellular NAD+ levels, inversely it's inhibition boosts intracellular NAD+ levels. Increased cellular NAD+ levels have been shown to inhibit immune responses. We hypothesize that the inhibition of CD38 enzymatic activity with a mAb could be an effective therapeutic approach to inhibit immune responses.

We developed AB181_185 a fully human heavy chain-only bispecific monoclonal anti-CD38 antibody that inhibits human CD38 enzymatic activity increasing cellular NAD^+ levels and without killing CD38+ cells. In this study we investigated the effect of AB181_185 administration to control acute GvHD in immune humanized NSG mice. Mice injected with human PBMCs developed GvHD in 16 days +/- 6 days and were euthanized (n=12), in contrast, all treated mice survived and had stable weight over a 50 days follow up (n=6, p=0.0005). Anti-CD38 mAb-treated mice showed significantly lower clinical score, liver leukocyte infiltration, numbers of human $CD45^+$ cells, T $CD4^+$ and T $CD8^+$ cells and $CD3^+CD69^+$ T cells but higher percentage of T $CD4^+FOXP3^+$ in spleen. Moreover, we found in treated mice a significant increase in the ratio of IFNγ/IL10.

Our results showed that AB181_185 efficiently prevents acute GvHD controlling T effector cell activation and preserving Tregs. Studies are on-going to analyze the role of NAD^+ on this effect. We propose that inhibition of CD38 enzymatic activity could be a new treatment for acute GVHD and, possibly, other immune mediated disease.

TH319. Accelerated Germinal Center Reaction by Targeting Vaccine Antigens to Major Histocompatibility Complex II

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Most vaccines induce protection by generating high levels of high affinity antibodies towards vaccine antigens. High affinity B cell clones emerge from germinal center (GC) reactions and enhancing the GC reaction is a prime objective in vaccine development. We demonstrate that targeting antigen to MHCII

on antigen presenting cells (APCs) increased the GC reaction, resulting in augmented levels of high affinity B cell clones in the GCs and ultimately more long-lived plasma cells in the bone marrow. Targeting APCs increased presentation of antigenic peptide on MHCII, and numbers of antigen reactive GC B cells and T follicular helper cells were elevated. Interestingly, the improved responses required cross-linking of BCR and MHCII in either cis or trans. B cells could function as efficient APCs when antigen was targeted to MHCII, and other APCs were redundant in vivo. The enhanced GC reaction induced by MHCII-targeting of antigen has clear implications for design of more efficient subunit vaccines, and particularly for influenza and HIV vaccines intended to induce broadly neutralizing antibodies.

TH361. A MAdCAM-tethered PD-1 Agonist Inhibits T cell Activation and Ameliorates Intestinal Inflammation

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T cell function is regulated by complex signaling networks of interconnected activators and inhibitors. Blockade of inhibitory receptors such as PD-1 has emerged as a novel treatment for multiple forms of cancer. One of the most common adverse events associated with blockade of the endogenous PD-1/PD-L1 pathway is the induction of autoimmune pathology, including in the intestine, demonstrating that PD-1 signals are necessary for normal immune homeostasis in humans. Given this body of clinical data, we sought to generate a PD-1 agonist antibody as a therapeutic target for autoimmune diseases. Rather than systemically modulating the immune response, we combined our PD-1 agonists with moieties targeting specific tissues to modulate activated T cells causing localized pathology. Here we demonstrate that a bifunctional MAdCAM-tethered PD-1 agonist molecule can signal through the PD-1 receptor and inhibit T cell effector functions in vitro. In vivo, the MAdCAM-tethered PD-1 agonist attenuates intestinal inflammation in a mouse model of xenogeneic graft versus host disease and provides a significant survival advantage compared with vehicle control animals. This work demonstrates that targeting PD-1 with an agonist antibody can inhibit undesirable immune responses in specific tissues.

TH363. PD-1 Protects Newly Activated Effector T Cells from Premature Restimulation-Induced Cell Death

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The immune system utilizes tightly regulated homeostasic processes to clear pathogens while avoiding host immunopathology. Restimulation-Induced Cell Death (RICD) is one such critical process in which activated T cells that receive subsequent antigenic stimulation reach a critical T Cell Receptor (TCR) signal threshold and commit to apoptosis, facilitating contraction of the T cell pool following pathogen

clearance. Programmed Cell Death -1 (PD-1) is a coinhibitory protein expressed in newly activated effector T cells which dampens TCR signal strength through recruitment of the phosphatase SHP2. Though its role in regulating exhaustion has been investigated thoroughly and blockade of this protein is being utilized as a cancer therapeutic, its role in regulating RICD remains unknown. *We hypothesized that PD-1 protects activated, expanding T cells from premature RICD.* In line with our hypothesis, decreased PD-1 presence or signaling increased RICD sensitivity in newly activated cells. In addition, pharmacological inhibition of SHP2 independently increased RICD, suggesting that PD-1 may protect newly activated cells from RICD via recruitment of SHP2. Downstream of SHP2, the transcription factor BATF has been associated with PD-1 activity; we found that knocking down BATF also increased RICD. We hypothesize that PD-1 recruitment of SHP2 alters BATF expression and ultimately changes expression patterns of apoptotic proteins that confer resistance to RICD during clonal expansion, which we are currently investigating through qPCR arrays. This work demonstrates that coinhibitory proteins like PD-1 are key modulators of immune system balance, helping to explain and potentially rectify unpleasant side effects of checkpoint blockade therapy.

TH445. Continued Bcl6 expression prevents the transdifferentiation of established Tfh cells into Th1 cells during acute viral infection

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T follicular helper (Tfh) cells are crucial for the establishment of germinal centers (GCs) and potent antibody responses. Nevertheless, the T cell-intrinsic factors that are required for the maintenance of already established Tfh cells and how they could be leveraged to modulate ongoing GCs remain largely unknown. Here, we used temporally guided gene ablation in $CD4^+$ T cells to dissect the contributions of the Tfh-associated chemokine receptor CXCR5 and the transcription factor Bcl6. Induced ablation of *Cxcr5* had minor effects on the function of established Tfh cells and *Cxcr5*-ablated cells still exhibited most features of CXCR5-positive Tfh cells. In contrast, continued Bcl6 expression was critical to maintain the GC Tfh cell phenotype and also the GC reaction. Importantly, T cell-specific *Bcl6* ablation during acute viral infection resulted in transdifferentiation of established Tfh into Th1 cells. These functional insights not only underline the high degree of plasticity of Tfh cells, but they also provide insights into how ongoing GC reactions may be modulated through boosting or restraining the function of Tfh cells in health and disease.

Immuno-oncology

F162. The Galectin-9/Tim-3 axis is associated with an immune-suppressive microenvironment in gastric cancer

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The development of immune checkpoint inhibitors (e.g. anti-PD1) has revolutionized cancer treatment. However, in gastric cancer (GC), only approximately 20% of patients show clinical benefit. An emerging checkpoint target is Tim-3, a membrane protein that enhances immunosuppression. Despite this, the expression and the role of its ligand Galectin-9 (Gal-9) in GC is still undeciphered. We hypothesize that Gal-9 could promote an immune-suppressive tumor microenvironment and that redundancy exists between checkpoint inhibitors.

We performed a bioinformatic analysis of gastric adenocarcinoma from the TCGA database to evaluate immune checkpoint expression patterns. Furthermore*, in vitro,* Gal-9 was transfected into GC cells (AGS) or recombinant Gal-9 was administrated. Eptihelial to Mesenchymal Transition (EMT) is a key step in metastasis, thus we evaluated levels of EMT markers by Western Blot. Furthermore, T cells and endothelial cells (HUVECs) were treated with conditioned media isolated from recombinant Gal-9 treated or Gal-9 transfected cancer cells to determine immune checkpoint expression. Given the observation that tumor endothelial cells express Gal-9, we evaluated the effect of this protein on angiogenesis and migration.

Results from TCGA analysis demonstrated a positive correlation between Gal-9, PDL-1 and Tim-3, which was consistent with *in vitro* experiments, where increased expression of Gal-9 resulted in increased PDL-1 and Tim-3 in AGS cell line. Furthermore, positive correlations were present between Gal-9 and Treg and T cell dysfunction markers. In addition, Gal-9 presence *in vitro* increased cancer cell invasion, EMT markers and angiogenesis.

These results suggest an association between Gal-9 and an immune suppressive microenvironment and GC progression.

F164. BET bromodomain proteins transduce AMPK signaling to regulate expression of multiple inhibitory receptors on T cell and NK cell subsets

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Co-morbid disorders of metabolism are rising among cancer patients. In Type 2 diabetes (T2D), a chronic inflammatory disease that associates with progression of several cancers, T cell subsets show abnormal polarization, metabolic impairment and elevated expression of inhibitory receptors (IR) in both

periphery and tumor infiltrates. T2D patients lose $CD8^+$ tumor surveillance, favoring escape of micrometastases from primary tumors. Yet existing approaches to immuno-oncology therapies have been tested in metabolically healthier cancer patients, rather than at safety net hospitals, where T2D sometimes affects up to half the population. The Bromodomain and Extra-Terminal (BET) proteins, comprising BRD2, BRD3 and BRD4, are transcriptional co-regulators of metabolism and are targets in cancer immunotherapy. We previously showed that individual BET proteins control PD-1 and PD-L1 expression, central to immunotherapy. Here we measured expression of PD-1, CTLA4, TIGIT and TIM-3 expression on human primary CD4+, CD8+, NK and NKT cell subsets, and manipulated metabolic signaling through 5' adenosine monophosphate-activated protein kinase (AMPK), a metabolically critical enzyme, dysregulated and routinely targeted by the first line T2D therapies such as metformin. We hypothesized that AMPK-dependent programs affect multiple immune lineages in a distinct, subset-specific manner that could explain the diverse actions of metformin in T2D patients. Small molecule BET inhibitors, including the pan-BET inhibitor JQ1 and the BRD4-selective degrader MZ-1, target multiple IR genes. We observed TIGIT and TIM3, but not PD1 and CTLA4, become BET-independent upon AMPK inhibition. Distinctive BET regulation of IRs could have value in cancer immunotherapy applications, particularly in T2D patients with cancer.

F169. Vδ1 T Cells with Cytotoxic Potential are TIGIT+ and Candidates for Adoptive Cell Therapy

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γδ T cells are MHC unrestricted and thus excellent candidates for 'off-the-shelf' pan-cancer adoptive cell immunotherapy, however, they have yet to demonstrate consistent clinical impact. Nevertheless, there has been an expansion in the number of companies and clinical trials focused on developing and testing γδ T cell therapy for cancer. Investigations are underway to identify alternative γδ T cells subsets for γδ T cell adoptive cell therapy. It is hypothesised that the Vδ1 subset are less susceptible to activation-induced exhaustion compared to Vγ9Vδ2 T cells. Here we characterised expression of inhibitory receptors on γδ T cells from cord blood, peripheral blood and endometrial tumours and identified TIGIT as a key 'checkpoint' on Vδ1 γδ T cells expressed on antigen experienced cells. We uncoupled the effect of stage of differentiation from TIGIT engagement and demonstrated it reduced Vδ1 proliferation. We showed peripheral blood TIGIT+Vδ1 cells have superior cytotoxic potential relative to TIGIT-Vδ1 cells. It has been revealed that the bioinformatic classification of the γδ T cell signature is indistinguishable from other immune cell signatures. Here, we use the γδ T cell specific gene *TRDV1*, unique to the Vδ1 chain of the γδ T cell receptor (TCR), to improve the intra-tumoral Vδ1 T cell signature. Using The Cancer Genome Atlas (TCGA) we demonstrated expression of TIGIT and TRDV1 was associated a cytotoxic gene signature and enhanced patient survival in some cancer types. We propose that TIGIT+Vδ1 T cells are anti-tumour candidates for adoptive cell immunotherapy, in combination with anti-TIGIT antibodies.

F198. NLRP3 regulates IL-4 expression in malignant CD4+ T cells of cutaneous T Lymphoma with an Implication in the disease progression

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Introduction. In cutaneous T cell lymphoma (CTCL), a dominance of Th2 profile related with the disease progression has been proposed. **Aim**. To evaluate IL-4 expression by malignant $CD4^+$ T cells in early stage of CTCL patients as well as the regulatory mechanism of IL-4 and its implication in the disease progression. **Material and Methods**. Skin biopsies of CTCL patients were collected. Malignant lymphocytes were identified by TOX expression. Proliferation and IL-4 expression were evaluated by flow cytometry. IL-4, NLRP3 and Karyopherin a2 expression was evaluated by immunofluorescence. NLRP3 expression and inflammosome assemble were inhibited using a specific siRNA and a pharmacologic inhibitor respectively. Recombinant IL-4 was added to evaluate IL-4 and TOX expression and NLRP3 localization. **Results**. The results show the presence of malignant $TOX^+/CD4^+/IL\text{-}4^+$ T cells in early-stage of CTCL patients. Interestingly, we found that in malignant cells NLRP3 is located in the nuclei and that reduction of NLRP3 expression or the inhibition of its assemble have an impact in the IL-4 expression. In addition, we observed that IL-4 amplified this mechanism increasing the expression of Th2 cytokines. We found that the mechanism of regulation of IL-4 mediated by NLRP3 is increased in tumor stage, which increases the malignant features in the $CD4^+$ T cells. **Conclusions**. The results suggest that NLRP3 is a key regulator of IL-4 expression in malignant $CD4^+$ T cells present in the skin lesions of CTCL patients and that this mechanism might have an important implication in the progression of the disease.

F214. Benchmarking of mouse tumor models against Human patterns of tumor-immune infiltration

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Although mouse models have provided unparalleled insights in tumor immunology, there is limited experimental data establishing robust correlation between the global immune profile of mouse and human tumors, in which these models often fail to accurately anticipate immunotherapy efficacy for humans. Such may be due the inability of current mouse models to capture pertinent and complex genetic and environmental variation contributing to human pathology. Elucidating this biological complexity will be vital for the generation of novel therapeutics.

To this end, we are benchmarking the immune profile of 14 common and exceptional mouse tumor models against Human data generated in the ImmunoProfiler Initiative at UCSF. This is done by performing standardized deep profiling of the immune infiltrate in these models by mass cytometry

(CyTOF) and single-cell RNA sequencing, and by studying human-relevant conditions including treatments with antibiotics, high-fat diet feeding and aging.

Once completed, our study will provide a strong rationale to the use of specific mouse models to study particular subtypes of patients and provide a major resource to deeply understand the biology of tumor-immune interactions.

F227. Functional Genomic Studies of Cancer Immune Evasion Using In Vitro and In Vivo CRISPR/Cas9 Genetic Screens

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Recent advances in cancer immunology have shed light on the interaction of the immune system with cancer. Expression of immune checkpoints by cancer cells allows their efficient immune evasion. The great potential of these findings is highlighted by approval of therapeutic antibodies blocking immune checkpoints. Development of novel immunotherapies constitutes an extremely promising clinical development, which was limited due to lack of reliable high-throughput technologies. With these in mind, I have carried out *in vitro* and *in vivo* CRISPR/Cas9 genome-wide screens. These, enable the identification of genes whose knockouts sensitise cancer cells to immune responses, while also allowing the characterisation of novel cancer immune evasion pathways. I have optimised these screens and have verified that genome-wide gRNA library coverage *in vivo* is possible in a model of colon cancer. Initial results indicate that disruption of antigen presentation pathways, interferon and NF-κB signalling are lethal to tumour cells *in vivo*. The identified candidate genes were validated *in vivo* and *in vitro* by designing targeted gRNA libraries. A number of significantly depleted and enriched genes have been identified, such as *Cd274* (PDL1) and *Ptpn2*, among others. Further work is focusing on investigating their mechanistic role by characterising the immune responses elicited by specific mutations. Finally, I'm planning to identify the main effector cells using flow cytometry along with single-cell RNAseq. I anticipate that these findings have the potential to advance our understanding of cancer immune evasion, while enabling the development of immunotherapies, by providing a framework for high-throughput target identification and validation.

F228. Studying the effect of immune checkpoint inhibitors on the human innate immune response

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Introduction: Cancer immune checkpoint inhibitor (CPI) therapy is frequently accompanied by immune-related adverse events (irAEs). The underlying mechanisms of these events are still poorly

understood. We set out to investigate serum cytokines/chemokines and innate immune cell frequencies and phenotypes before and after CPI treatment and whether these differ between patients who develop irAEs and those who do not.

Methods: The Pathobiology of Adverse Immune Reactions (PAIR) study recruits cancer patients requiring treatment with CPI therapy. Serum and PBMC were collected from PAIR patients prior to and during treatment with CPIs, including at times of toxicity. Serum cytokines/chemokines were analysed by 25-analyte bead array; PBMC were immunophenotyped by flow cytometry.

Results: Analysis of serum from 29 patients demonstrated that after a single dose of CPI, levels of IL-2R, IL-12, IFN-a, CXCL9 and CXCL10 were significantly increased in all patients, while IL-1RA was only significantly increased in those who developed toxicity. Significantly lower baseline serum levels of IL-2R were found in patients who developed toxicity compared to those who did not. Preliminary data suggest that there are alterations in certain innate immune cell populations upon checkpoint blockade and in relation to toxicity.

Conclusions: We describe an increase in various, mostly innate, cytokines/chemokines following the first dose of CPI, with differences between patients who develop irAEs. We are currently validating these findings in a second patient cohort, and investigating the immunophenotype of innate immune cell populations pre/post therapy.

Supported by Cancer Research UK, John Reece, and the NIHR-BRC at King's/GSTFT.

F233. Risk-Dependent Alterations in T Cell Exhaustion in Childhood Leukemia

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Risk stratification guiding management of acute leukemias in childhood is based on clinical features, and whether properties of the host immune system impact disease risk is not known. Here, we used high-dimensional single-cell analysis (mass cytometry and single-cell RNA sequencing) and functional studies to investigate the bone marrow immune cell repertoire of B-lineage acute lymphoblastic leukemia (B-ALL, n=36) patients, acute myelogenous leukemia (AML, n=28) patients, and healthy donors (n=11). Our studies reveal surprising evidence of chronic activation and exhaustion of bone marrow T cells in leukemia, with depletion of TCF1+ stem-like memory T cells and accumulation of T cells with a terminal effector/exhausted phenotype. Properties of immune cells identified distinct immune-based clusters correlating with disease risk in B-ALL. Analysis of T cell gene expression

revealed that high-risk individuals had enrichment of exhausted T cells relative to low-risk and healthy. Examination of the tumor cells revealed an association between tumor gene expression and immune risk; tumors with enriched stem-associated genes correlated with high-risk T cell phenotype. Our studies provide a comprehensive analysis of the immune landscape in childhood leukemia. They demonstrate that hierarchy of T cell exhaustion is established even in pediatric malignancies which are characterized by a much lower mutation burden relative to adult tumors. This link between the immune microenvironment and disease risk could be important to informing treatment decisions in the future, especially regarding immune therapies such as T cell redirection, which rely heavily on the efficacy of a patient's own immune repertoire.

F247. Novel Multi-specific Antibodies for Targeted Immune Modulation of Solid Tumors and Hematological malignancies

Udaya Rangaswamy[1], Brian Avanzino[1], Aarti Balasubramani[1], Starlynn Clarke[2], Kevin Dang[1], Laura Davison[1], Katherine Harris[1], Priya Ganesan[1], Brett Jorgensen[1], Yuping Li[1], Kyle Lorentsen[1], Harbani K Malik-Chaudhry[1], Harish Medlari[1], Duy Pham[1], Kirthana Prabhakar[1], Ute Schellenberger[3], Harshad Ugamraj[3], Wim Van Schooten[1], Roland Buelow[1], Nathan Trinklein[1] and Suhasini Iyer[1]

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Despite a revolutionary advance in the treatment of multiple cancer types owing to the success of antibodies targeting immune checkpoint blockade pathways, gaps remain in the understanding of mechanisms resulting in failure to treat certain cancers. There is an ever-growing need for novel modalities to harness the immune system to facilitate better cancer treatments.

UniRats™ are novel transgenic rats used to generate human HCAbs (Heavy Chain only antibodies), termed UniAbs (Clarke S *et al*, Frontiers in Immunology, Jan 2019). Using a high-throughput discovery platform, we can rapidly generate a distinct set of antibodies against any antigen. UniAbs serve as building blocks for development of multi-specific antibodies with desired functional outcomes. The versatility of the UniAbs enables creation of multi-specific antibody moieties namely T-cell redirecting antibodies, antibodies against costimulatory molecules or antibodies targeting multimeric cell surface receptors, in a plug-and-play fashion. As an example, our unique T cell redirection platform utilizes an anti-CD3 arm which, when combined with an anti-tumor associated antigen arm (anti-TAA), results in efficient tumor lysis with minimal cytokine release. Using a case study in solid tumors, we will highlight the modular nature of this platform for rapid therapeutic development.

F264. Immune Profiling of the Tumor Microenvironment Using Multiplexed Ion Beam Imaging (MIBI)

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Traditional imaging platforms are limited in their ability to identify both the cell types present in the tumor microenvironment and the spatial relationship between immune and cancerous cells. To address this, MIBI has been developed to image up to 40 markers at single cell resolution.

Staining of 10 non-small-cell lung carcinoma (NSCLC) formalin-fixed paraffin embedded tissue samples was performed similarly to traditional Immunohistochemistry (IHC) except that a panel of 22 metal labeled antibodies were applied to the slide simultaneously. The tissue was imaged at subcellular resolution using an ion beam and time-of-flight secondary ion mass spectrometry (ToF-SIMS). The masses of detected species were then assigned to target biomolecules given the unique label of each antibody. Multi-step processing and segmentation utilizing highly expressed nuclear, membrane, and cytoplasmic markers were performed to accurately determine cell boundaries and enable quantitative analyses marker expression, cell classification, and the spatial relationships between cells types. Results comparing the abundance of tumor cells, vascular cells, and immune cell subsets between samples identified “cold” tumors with few immune cells and inflamed samples that varied in the type of immune populations present. Immune checkpoint markers (LAG3, PD-1, PD-L1, TIM-3) were also quantified at single cell resolution. In conclusion, this study shows that MIBI offers high-parameter capability necessary to (1) identify the wide range of cell types present within the complex tumor immune landscape, (2) the spatial relationships between these different cell populations, and (3) the expression status of key immunoregulatory proteins.

F271. Leveraging gene signatures to define immune classes across human tumor

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In Immunoprofiler Consortium (IPI) at UCSF, and our aim is to understand the immunological basis of cancer and its effects on the efficacy of treatment. Immunotherapy and especially immune checkpoint blockade have shown outstanding results in the treatment of cancer but only in 20% of treated patients. This low efficacy could be due to variability of the TIME between different patients or to the fact that treatment studies have been solely conducted in mouse models. In order to understand the nature of the immune response to cancer we have established a pipeline to immunophenotype a broad range of solid tumors. We combine transcriptomics, single cells analysis, infiltrate composition and to find patterns that are conserved across many different types of human tumors (e.g. Lung, Bladder, Head and Neck, Ovarian, Colorectal, etc.) in order to identify disease types and immunological tumor states.

F290. An Engineered Allogeneic Artificial Antigen Presenting Red Cell Therapeutic, RTX-321, Promotes Antigen-Specific T Cell Expansion and Anti-Tumor Activity

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The wider adoption of antigen-specific therapies, such as CAR-T and cancer vaccines, has been limited by significant toxicity, limited effectiveness in solid tumors and difficulties in manufacturing at scale. To address these limitations, Rubius Therapeutics has genetically engineered red cells to create an allogeneic artificial antigen-presenting cell (aAPC), RTX-321, that presents an HPV E7 peptide on MHC I, 4-1BBL and IL-12 to mimic the human immunobiology of T cell-APC interactions.

RTX-321 expanded HPV-specific TCR-transduced primary human CD8 T cells with an effector phenotype. To determine in vivo efficacy, a surrogate murine red cell (mRBC-321) was designed with surface conjugated murine MHC I-OVA, 4-1BBL and IL-12. mRBC-321 treatment resulted in 15/16 tumor regressions and 11/16 cures of EG7.OVA tumors with OT1 adoptive transfer. mRBC-321 promotes antigen-specific T cells expansion and effector function in addition to favorable non-antigen-specific immune modulations in the tumor. Cured mice were resistant to re-challenge with EG7.OVA, which correlated with OT1 and endogenous OVA-specific T cell expansion. Challenge with the parental EL4 tumors demonstrated 3/7 delays and 3/7 cures, suggesting antigen spreading. Importantly, in mice without OT1 transfer, mRBC-321 significantly delayed EG7.OVA tumor growth and extended survival.

These results support the planned IND filing of RTX-321 for the treatment of HPV16+ advanced solid tumors by the end of 2020. Beyond application to HPV-positive tumors, the allogeneic RCT-aAPC platform is highly modular and is being developed for application to shared and personal cancer neoantigens and additionally offers the potential for the treatment of autoimmune and inflammatory diseases.

F304. Exploration of Immune Checkpoints Ligands in Exosome-Enriched Fractions During the Progression of the Cervical Cancer

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Background. Cervical cancer represents the third most commonly diagnosed cancer in women worldwide. Among the most novel therapeutic strategies for the treatment of cancers is the blockade of important immune checkpoint molecules expressed by T and NK cells, such as PD-L1 or TIGIT. These receptors negatively regulate the activation upon the interaction with their ligands PD-L1 and

CD155/Nectin-2, respectively; conversely, NKG2D positively regulates the activation after engagement to MICA/B. However, tumor cells can release these ligands via exosomes promoting different physiological consequences. **Objective.** To determine whether these ligands are released to the systemic circulation in a soluble form or confined to exosome fractions following the progression of the cervical cancer. **Methods.** Exosomes were isolated from serum of patients with intraepithelial lesions or cervical cancer (a group of healthy women was included as a control). By Western blot, we identified T and NK cell ligands in total sera, exosome-free fractions and exosome-enriched fractions. **Results.** Our results showed different molecular weights of PD-L1, MICA/B, CD155 and Nectin-2, which were found either in exosomes or in exosome-free fraction, with a similar pattern in all groups. In the case of PD-L1, the most abundant band was between 25-30 kDa, followed by a 100 kDa band. The 100 kDa band was absent in the exosome-enriched fraction, though the other bands were present. It is possible that the presence of the 25-30 kDa bands, which were confined to exosomes, might be a biomarker to identify patients most likely to respond well to anti-PD-L1 immunotherapy.

F339. Peripheral T cell expansion predicts tumor infiltration and clinical response

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Despite the resounding clinical success in cancer treatment by antibodies that block the interaction of PD-1 with its ligand PD-L1 [Mellman 2011], key unknowns remain concerning the mechanisms responsible. A major limitation to understanding the origin and fate of T cells in tumor immunity is the lack of quantitative information on the distribution of individual T cell clonotypes in cancer patients. Here we performed deep single-cell sequencing of RNA and T cell receptors in 14 cancer patients across four different cancer types, from surgical resections of their tumor and normal adjacent tissue (NAT), and in 4 patients, from peripheral blood. We obtained 330 million mRNA transcripts across 141,623 T cells, of which 99,788 had clonotypes. T cells were grouped into 56,975 distinct clonotypes by matching CDR3 regions, which allowed us to measure clonal expansion and track clonal lineages across tissues. We find clear evidence of clonotypic expansion of T effector-like cells not only within the tumor but also in NAT. Patients with gene signatures of such clonotypic expansion respond best to anti-PD-L1 therapy. Importantly, expanded clonotypes found in the tumor and NAT can also typically be detected in peripheral blood, suggesting a convenient approach to patient identification. Analysis of our data along with several external datasets suggests that intratumoral T cells, especially in responsive patients, are continuously replenished with fresh, non-exhausted replacement cells from sites outside of the tumor, suggesting continued activity of the cancer immunity cycle in these patients, the acceleration of which may be associated with clinical response.

F414. The Generation of “Off-the-Shelf” CD19-Chimeric Antigen Receptor (CAR) T Cells Using Umbilical Cord Blood as Source of T lymphocytes.

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Background: T lymphocytes through the expression of chimeric antigen receptors (CAR) can be redirected to tumors and showed unprecedented clinical success for pediatric and adult B cell malignancies. Umbilical cord blood (UCB) represents a promising source of T cells for the generation of "off-the-shelf" CAR-T cells, replacing the manufacturing in autologous setting. The aim of this study is to prove that CAR-T cells can be efficiently generated from UCB and perform a deep characterization of these CAR-T cells. **Methods:** UCBs samples were obtained from Sidra Medicine. T-Lymphocytes were negatively enriched by magnetic selection (Miltenyi Biotech) and activated by agonistic anti-CD3 and anti-CD28 mAb (either beads, Dynabeads or TransAct (Miltenyi Biotech). T cell transduction was performed *in vitro* with lentiviral vectors (CD19-CD28z or CD19-4-1BBz CARs), Deep phenotype analyses and functional characterization of CAR-T cells was performed. **Results:** T lymphocytes from either UCB or PBMC showed high efficiency in expressing the CARs (40-70% of positive cells). Preferential selection of early differentiation/central memory T cells was observed in UCB- vs. PBMC-CAR-T cells (CD45RA+CCR7$^+$CD28$^+$CD27$^+$CD137$^+$CD62L$^+$). Efficient *in vitro* expansion of UCB-CAR-T cells was observed with 26-56-fold increase and the obtained cells can reach up to 4x10^{8}cells. Anti-tumor-specific reactivity was observed following the co-culture of CD19-CAR-T cells with CD19$^+$target cells. IFN-g, granzyme B and perforin were secreted by T cells and detected through EliSpot and FluoroSpot assays upon engagement of CD19-CAR. The transcriptomic profile of UCB-CAR-T was assessed upon antigen-specific stimulation. **Conclusions:** UCB represent efficient source of T cells for the generation of "off-the-shelf" CAR-T cells.

F425. The Single Cell Landscape Defining Checkpoint Inhibitor Colitis Opens a Window Into Immune Dysregulation in The Human Colon

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Antibodies against CTLA-4 and PD-1 have revolutionized metastatic solid tumor treatment. However, their use is limited by immune-related adverse events. The gastrointestinal tract is a frequent target of this immune attack seen in approximately 45% of patients on dual PD-1 and CTLA-4 blockade. To better understand these potent immune responses, we analyzed ~180,000 single cells generated from endoscopic colon biopsies by RNA sequencing (scRNAseq) of epithelial, stroma, and immune cell populations paired with T cell receptor (TCR) and B cell receptor profiling. Our cohort comprised 13 patients with checkpoint therapy colitis (irColitis), 10 patients on checkpoint therapy without colitis, and 8 healthy controls. Compared to controls, patients with irColitis displayed a marked expansion of mucosal T regulatory cells and tissue resident CD8 T cells with multiple features of "dysfunction," cytotoxicity, and cell cycling. In addition, resident CD8 T Cells demonstrated increased TCR diversification. Epithelial cells from patients affected by colitis displayed defects in epithelial stem cells

accompanied by a strong interferon signature. To better understand the immunologic context of irColitis, we generated additional scRNAseq libraries from 4 patients with microscopic colitis. We then integrated our data with a published set of 350,000 cells from 18 patients with ulcerative colitis and 12 healthy controls to generate a dataset of 550,000 cells from which we could infer biological commonalities with inflammatory bowel disease. Together these data highlight the molecular mechanisms driving irColitis and shed light on the specific role of CTLA-4 and PD-1 signaling in maintaining gastrointestinal immune tolerance.

F431. Increased NK cell-mediated lysis of BRAF inhibitor resistant cells.

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Targeted therapies and immunotherapies are first-line treatments for patients with advanced melanoma. BRAF and MEK inhibition leads to a 70% response rate in patients with advanced melanoma with a $BRAF^{V600EI_K}$ mutation. However, acquired resistance occurs in the majority of patients, leading to relapse. Immunotherapies activating immune cytotoxic effectors induce long lasting responses in 30% of patients. In that context, combination of targeted therapies with immunotherapy is a promising approach. We considered boosting Natural Killer (NK) cell tumor immunosurveillance, as melanoma cells express stress-induced molecules and activate NK cell-lysis.

We have generated vemurafenib (a BRAF inihibitor) resistant (R) cells from $BRAF^{V600E}$ SK28 and M14 sensitive (S) melanoma cell lines and investigated how resistance interferes with immunogenicity to NK cells. Vemurafenib resistance was accompanied by modulation of NK ligands binding NKG2D activating receptor. Resistant melanoma cells lines (SK28R and M14R) induced higher NK degranulation, IFNγ secretion and were more efficiently lyzed by donor and patient NK cells. In addition, SK28R showed increased TRAIL-RII expression and TRAIL-induced apoptosis.

Soluble NKG2D ligands known to regulate the receptor function have been associated to cancer progression. Thus, we determined serum levels of several soluble ligands in 61 melanoma patients at baseline and 6 months M post treatment with targeted therapies or immunotherapies. We found that levels of soluble NK ligands (MICA, B7H6) were correlated with clinical response and may predict the response to immune checkpoint in melanoma patients.

F442. A Novel Bioluminescent Immunoassay Platform for Sensitive and Homogeneous Analyte Detection Using Labeled Antibodies

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Promega, Madison, WI

NanoLuc® Binary Technology (NanoBiT®), a two-part complementation system based on NanoLuc luciferase, is a proven technology for analyzing proteins at a cellular level. NanoBiT is comprised of an 11-amino acid subunit (low-affinity SmBiT or high-affinity HiBiT) that binds to its cognate large subunit partner (LgBiT) to form a bright luciferase that produces light when furimazine is added. We are building NanoBiT proximity immunoassays where complementary antibodies (or other affinity reagents) are labeled with NanoBiT subunits such that binding to analyte brings SmBiT and LgBiT into proximity, thereby producing signal proportional to analyte levels. This homogeneous detection chemistry has several advantages, including simple, add-and-read protocols, no requirement for sample transfer, no washes, and a broad linear dynamic range mitigating the need for sample dilutions. Moreover, time to assay completion is < 30 to < 90 minutes, depending on the specific assay. In development are assays for detection of cytokines (e.g., IL-1β), metabolic targets (e.g., Insulin), FcRn binding, cellular pathway analyses (total and phospho-protein levels), as well as labeling kits to build your own Lumit immunoassays.

F443. Cell-based Reporter Bioassays for Development of Fc-functional and Fc-silent SIRPalpha/CD47 Checkpoint Inhibitors

Kyle Hooper, Frank Fan, Mei Cong, Zhijie Jey Cheng, Jonathan Mitchell, Jamison Grailer and Jim Hartnett

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CD47 interacts with SIRPα on myeloid cells to deliver a “don’t eat me” signal that inhibits phagocytosis. SIRPα/CD47 blockade promotes phagocytosis of tumor cells *in vitro* and anti-tumor activity *in vivo*. Pre-clinical data have sparked development of SIRPα/CD47 inhibitors and a need for robust functional assays to measure the activity of these drug candidates. SIRPα/CD47 inhibitor activity is typically assessed via *in vitro* phagocytosis assays using imaging or flow cytometry. These assays use primary monocyte-derived macrophages and are prone to donor variability, low-throughput, and difficult to implement in drug development settings. Additionally, many SIRPα/CD47 inhibitors are designed to minimize Fc function to enhance safety and are thus incapable of driving phagocytosis directly. To overcome these limitations, we have developed reporter-based bioassays to measure Fc-functional and Fc-silent SIRPα/CD47 inhibitor activity. These bioassays utilize a SIRPα-positive monocyte effector cell-line that expresses multiple Fc gamma receptors (FcγRs) and an FcγR-responsive NanoLuc luciferase reporter that is inhibited by SIRPα/CD47 interaction upon co-culture of SIRPα effector cells with CD47-positive target cells. CD47 inhibitors with Fc functional activity simultaneously disrupt the SIRPα/CD47 interaction and engage FcγRs on the SIRPα effector cells, resulting in NanoLuc reporter activation. To measure Fc-silent inhibitors, we have engineered a second CD47 target cell-line which provides a constitutive activating stimulus to SIRPα effector cells that is restricted by SIRPα/CD47 interaction. Addition of Fc-silent SIRPα/CD47 blockers releases inhibition by SIRPα/CD47, resulting in NanoLuc reporter activation. These reporter bioassays provide a robust, high-throughput platform to facilitate discovery and development of diverse SIRPα/CD47 checkpoint inhibitors.

TH12. Antigen-cross presentation promotes development of terminally differentiated CD8 T cells in young individuals

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Cancer immunotherapeutic approaches that rely on endogenous T cell responses have shown limited success in children, a pattern attributed to the low mutational burden of pediatric tumors. Here, we report that CD8 T cells isolated from a diverse set of pediatric solid tumors are enriched for an antigen-experienced phenotype. The limited ability of immune checkpoint blockade therapy (ICBT) to trigger anti-tumor responses in children, despite the presence of activated CD8 T cells, prompted us to explore alternative underlying mechanisms restricting anti-tumor responses. Using a novel mouse tumor model that expresses a well-characterized epitope coupled to an mCherry marker, we identified antigen cross-presentation by tumor-infiltrating myeloid cells as a key regulator of CD8 T cell effector function in tumors. Strikingly, age-related changes in the TME resulted in a skewing of the CD8 T cell effector fate toward a terminally differentiated state in young tumor-bearing mice. Profiling of tumor-infiltrating antigen-presenting cells by scRNAseq revealed a proinflammatory M1 macrophage polarization in young tumors but a predominant M2 “wound healing” response in adult tumors. Consistent with our mouse findings, analysis of immune infiltrates from human pediatric solid tumors revealed a strong correlation between the expression of PDL1 on myeloid cells and enrichment of tumor-associated CD8 T cells with an exhaustion phenotype. Collectively, these data indicate that the “young” microenvironment of an actively developing tissue/individual contributes to the generation of an immune response skewed towards a terminally differentiated state with limited plasticity, thus narrowing the window for immunotherapeutic interventions.

TH24. Transcriptional Analysis of Clinical-grade, Allo-antigen Specific Type 1 Regulatory T cells Reveals An Additional, IL-10-independent Mechanism of Suppression

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Type 1 regulatory T cells (Tr1) are peripherally inducible regulatory T cells crucial for peripheral tolerance. Alloantigen-specific Tr1 can be used to prevent graft-versus-host disease (GvHD) post allo-HSCT and improve its successful outcome in hematological malignancies. We have designed an *in vitro* method to induce Tr1 by stimulating donor-derived T cells with patient allogeneic tolerogenic dendritic cells. The resulting cell product, called T-allo10, suppress host-reactive effector T cells (Teff), but not Teff responses to other antigens, including responses to tumor antigens. Thus, alloantigen-specific Tr1 cells present in the T-allo10 product (Tr1-Tallo10) could prevent GvHD without impairing anti-tumor

effect or host defense, which is a critical unmet goal in allo-HSCT. The T-allo10 cell product is currently being tested at Stanford in a phase I/II clinical trial (NCT03198234).

Tr1 co-express surface LAG3 and CD49b, low or no FOXP3, and secrete of high levels of IL-10, which is considered key for their suppressive function. We performed a comprehensive molecular characterization of the TCR repertoires and transcriptomes of alloantigen-specific $LAG3^{+}CD49b^{+}$ Tr1-T-allo10 and $LAG3^{-}CD49b^{-}$ Teff. Tr1-T-allo10 had a restricted TCR repertoire, and expressed multiple genes encoding co-inhibitory molecules and other genes ordinarily associated with the $FOXP3^{+}$ thymic-derived regulatory T cells (nTregs). For some of these genes, we confirmed their functional role in Tr1-mediated suppression of Teff, indicating that Tr1, besides IL-10 secretion, utilize additional pathways to suppress immune responses. Altogether, this data suggests that Tr1, although developmentally different to nTregs, acquire similar transcriptional programs and utilize similar mechanisms of immune regulation.

TH42. Nanoparticle-enabled In Vivo Innate Immune Stimulation Breaks Peripheral Tolerance and Activates Endogenous Tumor Infiltrating T cells with Broad Antigen Specificities

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Tumors are often infiltrated by T lymphocytes recognizing either self or mutated antigens, but are generally inactive, although they often show signs of prior clonal expansion. Hypothesizing that their arrest may be due to peripheral tolerance, we formulated nanoparticles containing innate immune stimulants that we found were sufficient to activate self-specific $CD8^{+}$ T cells and injected them into two different mouse tumor models, B16F10 and MC38. This robustly activated and/or expanded antigen-specific $CD8^{+}$ tumor infiltrating T cells, along with a decrease in regulatory $CD4^{+}$ T cells, and an increase in IL-17 producers, and resulted in significant tumor growth retardation or elimination and immune memory in surviving mice. Furthermore, nanoparticles modified to stimulate human T cells enabled the robust activation of the endogenous T cells in patient-derived tumor organoids. These results indicate that breaking peripheral tolerance without regard to what specific antigens are involved creates a novel pathway for cancer immunotherapy.

TH50. Interleukin-6 Blockade Improves Response to Anti-PD-L1 Therapy by Promoting CD8+ T Cell Effector Function

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Checkpoint immunotherapy (CPI) is an effective therapeutic approach for multiple forms of cancer, but treatment resistance remains a major challenge. To identify biomarkers of clinical response to blockade of PD-L1 (programmed death ligand-1), we analyzed plasma from patients treated with atezolizumab (anti-PD-L1) in clinical trials of advanced urothelial bladder carcinoma (IMvigor210/211), renal cell carcinoma (IMmotion150), and triple-negative breast cancer (PCD4989g). Remarkably, high plasma IL-6 (interleukin-6) concentration was significantly associated with poor clinical response in all three studies. Notably, single-cell RNA-sequencing of peripheral blood mononuclear cells from patients with bladder cancer revealed reduced $CD8^+$ T cell effector function in patients with high plasma IL-6. Following anti-CD3/CD28 activation of mouse $CD8^+$ T cells *in vitro*, IL-6 inhibited the acquisition of potent effector function, including reduced cytokine production (e.g. IFN-γ and TNF) and impaired cytotoxicity, without significantly affecting cell proliferation. In syngeneic mouse tumor models (EMT6 mammary carcinoma and CT26 colon carcinoma) blockade of the IL-6 receptor (IL6R) synergized with anti-PD-L1 therapy to drive potent anti-tumor $CD8^+$ T cell responses and superior control of tumor growth compared to anti-PD-L1 treatment alone. Thus, IL-6 may be a useful biomarker for anti-PD-L1 clinical activity, and may contribute mechanistically to therapeutic resistance. Because IL-6 is a clinically approved therapeutic target, clinical trials to investigate blockade of IL-6 or IL6R in combination with CPI could be rapidly implemented, with potential for increased therapeutic benefit in multiple forms of cancer.

TH75. Optimizing TNFR2 Antagonism for Cancer Immunotherapy: Antibody Structure and TNFR2 Density on Target Cells Drive Tumor Microenvironment Specificity

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Most current immunotherapies lack Treg or tumor specificity. Tumor necrosis factor receptor 2 (TNFR2) antibody antagonism may be an attractive cancer immunotherapy due to its tumor microenvironment specificity. Human TNFR2 is massively overexpressed on tumor-residing Tregs, underexpressed on T effectors (Teffs) and found directly on some cancer cells. We screened 791 human tumor cell lines from diverse cancer tissues, finding frequent and aberrant TNFR2 expression. Using three cell-based tumor microenvironment assays, we tested a novel human-directed TNFR2 antagonist placed on different human isoform frameworks. The TNFR2 antagonist killed Tregs and cancer cells on the human chimeric IgG2 but not IgG1 isoform, without killing Teffs. The antagonist worked best against more rapidly proliferating Tregs or tumor cells, providing a reliable test cell line for further antibody development. Using mutagenesis techniques, we tested different mutations to our novel TNFR2 antagonist on human chimeric structures. Chimeric human IgG2 isoform were functionally superior to chimeric IgG1 isoform in the assays. The natural variability of the IgG2 TNFR2 antibody hinge had to be

stabilized with mutations for consistent tumor targeting and antibody purity. Narrowing the distance between antibody arms diminished activity. These findings suggest that the ideal TNFR2 antagonists are the human IgG2 isoform, have hinge stabilization, and have wider separation of antibody arms to bind to newly synthesized TNFR2 on rapidly growing tumor cells. When bound to TNFR2, antagonistic antibodies with these characteristics form a non-signaling cell surface dimer which functions as an effective immunotherapy with high tumor microenvironment specificity.

TH80. TCF-1 deficiency recapitulates the enhanced tumor promoting properties of Tregs in colon cancer

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We described earlier that Tregs in colon cancer gain pro-inflammatory properties but maintain their ability to suppress T-cells. These properties were attributed to increased β-catenin signaling. More recently TCF-1, the DNA binding partner of β-catenin was reported to be essential for maintaining Treg immunosuppressive functions. Binding of b-catenin toTcf-1 reverses the function of TCF-1 from a transcriptional repressor to an activator. Therefore, we argued that genetic ablation of Tcf-1 in Tregs could activate genes whose expression is enhanced by b-catenin activity in colon cancer and render the Tregs proinflammatory and tumor promoting. Here we combined bulk RNA-Seq and single cell RNA-seq with functional assays to establish how Treg suppressive functions are altered by loss of TCF-1. In WT mice, we identified naïve Treg populations, and traced their progression to activated Tregs. Distint activated Treg clusters were identified, and named based on expression of characteristic genes and pathways, as IFN, Tgfr, Rorc, Gata3, and Mif. Tcf-1 deficiency enhanced TH17 signaling in nearly all Treg clusters, but also enhanced TGFβ signaling, promoted Treg activation and expansion. Remarkably, TCF-1 deficient Tregs were more potent in suppressing T-cell proliferation and cytotoxicity, but were at the same time compromised in suppressing inflammation. These combined attributes enhanced the tumor promoting properties of Tregs in mice genetically predisposed to cancer. Therefore, TCF-1 deficiency reproduces the enhanced tumor promoting properties of Tregs in colon cancer, by enhancing Treg suppression of tumor immunosruveillance while compromising the Treg ability to suppress inflammation.

TH119. Acute loss of Treg function upon stimulation with 4-1BB or TNFR2-encoding CARs

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Introduction: Chimeric Antigen Receptors (CARs) can re-direct T-cell specificity and stimulate via a combination of co-stimulatory molecules and signalling domains. We previously developed a CAR specific for HLA-A2 and showed that CAR-Tregs had improved suppressive function when tested in transplantation models. However, this work employed a CD28-based CAR and it was not known if this

design was optimal for Tregs. Our aim was to study how CARs encoding alternative costimulatory domains affected Treg function.

Methods: CARs encoding one of 10 different costimulatory domains were generated. Transduced Tregs were tested in vitro and in vivo. For in vitro assays, CAR-Tregs were stimulated with HLA-A2-coated beads then cell expansion, phenotype and methylation of *FOXP3* TSDR region were determined. For in vivo function, a xenogeneic graft-versus-host disease (GVHD) model was used.

Results: Wild-type CD28-CAR was superior to all other CARs in vivo. Surprisingly, 4-1BB- or TNFR2-CARs Tregs were inferior to antigen-non-specific Treg controls. 4-1BB- or TNFR2-CARs stimulated rapid proliferation in vitro, whereas the CD28-CAR stimulated an intermediate proliferation. Whereas no differences were observed in FOXP3 expression, stimulation via 4-1BB- or TNFR2-CARs stimulated loss of Helios expression. 4-1BB- or TNFR2-CARs Tregs also re-methylated the *FOXP3* TSDR region, which was directly correlated with Helios expression.

Conclusions: This co-receptor domain CAR variants comparison in Tregs revealed key features of CAR-Treg biology and defined in vitro assays correlated with in vivo function. These data show that Tregs have distinct requirements for optimal CAR-mediated function and reveal new aspects that can be used to further optimize CAR design.

TH125. Identification of Pro-inflammatory Gene Variants in Triple Negative Breast Cancer Patients.

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Upregulation of the IL-4 receptor (IL-4R) is observed in various tumours and tumour associated macrophages (TAMs). This receptor binds to IL-4, a Th2 cytokine that is secreted by cancer cells and is responsible for the polarization of TAMs into non-classical (M2) macrophages that are believed to favour tumour progression. Triple-negative breast cancer (TNBC) is a heterogeneous disease often characterized by aggressive biology and poor prognosis due to its high capacity for invasion and lack of targeted therapy. We hypothesised that chronic inflammation may play a role in aggressive forms of TNBC seen in Kenyan patients. DNA extracted from saliva samples of 5 Kenyan patients with TNBC were analyzed using whole-exome sequencing (WES) on the Ion Proton. Bioinformatics tools (Varsome and ClinVaR) were used to predict the functional effects for variants of interest. Three out of five patients carried a missense mutation in IL-4R gene (c.A223G:p.I75V). Polymorphism of this receptor results in increased IgE production by tumour cells in a manner like an allergic response. Aggressive nature of TNBC could be associated with an asymptomatic atopic state of the immune response following IL-4R polymorphism that is known to initiate a less potent Th2 polarised immune response. Further investigations regarding the immune profiles of asymptomatic atopic individuals may provide additional clues about the biological mechanisms underlying aggressive forms of breast cancer in Kenyan patients.

TH130. Targeting TMEM176A enhances venetoclax-induced apoptosis through inflammasome activation in leukemic cells.

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Inhibition of the anti-apoptotic protein Bcl-2 with Venetoclax achieves high rates of durable complete responses in hematological malignancies. Nevertheless, recurrence is observed over months and years. Thus, unravelling the death mechanisms triggered by Venetoclax is critical to characterizing the resistance pathways. Along this line, Bcl-2 is known to inhibit inflammasome/Caspase-1-dependent cleavage of pore-forming protein Gasdermin D (GSDMD). Moreover, we have previously shown that inflammasome/Caspase-1 activation is inhibited by the intracellular cation channels TMEM176A/B in DCs and macrophages through ionic mechanisms. Thus, TMEM176A/B may impair venetoclax-induced cell death by inhibiting inflammasome/Caspase-1 activation. We first characterized TMEM176A/B as a molecular target in chronic lymphocytic leukemia (CLL). We found that TMEM176B mRNA expression in peripheral blood from CLL patients was associated with worse overall survival (n=298; p< 0.0001). Moreover, leukemic cells in peripheral blood from progressor patients expressed significantly higher TMEM176A protein levels than indolent patients (n=14; p< 0.01). CLL cells from indolent patients showed increased activated Caspase-1 versus progressors. Furthermore, TMEM176A expression was negatively correlated (R= 0.78; p= 0.001) with cleaved GSDMD. Accordingly, TMEM176A knock down as well as pharmacologic inhibition with AP-017 triggered Caspase-1 dependent cell death of primary CLL cells. On the other hand, Bcl-2 inhibition with Venetoclax also induced GSDMD cleavage. Importantly, AP-017 reinforced Venetoclax-induced cell death in human primary CLL cells and in murine TCL1 leukemic cells in vitro. In vivo, AP-017 + Venetoclax therapy significantly controlled TCL1 progression versus monotherapies. In conclusion, TMEM176A arises as a potential resistance factor to Venetoclax therapy by inhibiting inflammasome activation.

TH133. Limiting Oxidative DNA Damage Reduces Microbe-Induced Colitis Associated Colorectal Cancer

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Patients with inflammatory bowel disease have a greatly increased risk of developing colitis-associated colon cancer (CAC); however, the basis for inflammation-induced genetic damage requisite for neoplasia is unclear. Using three models of CAC, we found that sustained inflammation triggers the DNA lesion 8-oxoguanine. Strikingly, antioxidants or compounds that inhibit iNOS reduce 8-oxoguanine and polyps in CAC models. Because the mismatch repair (MMR) system repairs 8-oxoguanine and is frequently defective in colorectal cancer (CRC), we tested whether 8-oxoguanine is an oncogenic mediator in a Lynch syndrome (MMR-deficient) model. We show that microbiota generates an accumulation of 8-oxoguanine lesions in MMR-deficient colon tissue. Accordingly, we found that 8-oxoguanine was elevated in neoplastic tissue of Lynch syndrome patients compared to matched

untransformed tissue or non-Lynch syndrome neoplastic tissue. While antioxidants reduced 8-oxoguanine, they did not reduce CRC in Lynch syndrome models. Hence, microbe-induced oxidative/nitrosative DNA damage play causative roles in inflammatory CRC models, but not in Lynch syndrome models.

TH136. Isolation of Human CD45+ Leukocytes from Tissues and Human Tumor Xenografts in Humanized Mice

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The study of immune cell function in non-lymphoid tissue and tumors promises to elucidate novel strategies to treat immune disorders, infectious diseases, and cancer. To address the challenge of isolating leukocytes from complex and variable tissues and tumors, we have developed a new protocol to isolate particle-free, human CD45+ leukocytes. Using the EasySep™ Release Human CD45 Positive Selection Kit, leukocytes are labeled with antibody complexes linked to magnetic particles and separated using an EasySep™ magnet. The magnetic particles are then removed from the desired cells by resuspension in EasySep™ Release Buffer followed by a final magnetic separation.

To assess performance, NRG-3GS mice were engrafted with human CD34+ cells followed by xenotransplantion with human breast (MDA-MB-231) or ovarian (SKOV3) cancer cell lines. In humanized mouse lungs, bone marrow and spleen, the starting and isolated human CD45+ frequency ranges were 6.0 - 57.2% and 90.9 - 99.4%, respectively (n = 3). Starting with human tumor xenografts, tumor infiltrating leukocytes were enriched from a starting range of 0.4 - 18.0% to 76.6 - 92.7% (n = 4). The final immune cell frequencies were representative of the starting population, and further separation of immune subsets can be achieved with additional downstream isolation.

Humanized mouse models of clinical disease are instrumental in furthering our understanding of complex mechanisms of disease progression and resolution. This new kit for the isolation of human immune cells from tissues and tumors will facilitate further examination of the roles of immunity in disease and the evaluation of immune-based treatment strategies.

TH141. Distinct tumor microenvironments determine clinical fate and response to immunotherapy in Hepatitis B virus-related Hepatocellular carcinoma

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Chronic inflammation induced by chronic hepatitis B virus (HBV) infection increases the risk of hepatocellular carcinoma (HCC). However, little is known about the immune landscape of HBV-related HCC and its influence on the design of effective cancer immunotherapeutics.

In-depth interrogation of the immune landscapes by Cytometry by Time-of-Flight (CyTOF) showed that regulatory T cells (T_{REG}) and $CD8^+$ resident memory T cells (T_{RM}) were enriched in HBV-related HCC whereas $Tim\text{-}3^+CD8^+$ T cells and $CD244^+$ natural killer cells were enriched in non-viral-related HCC. Next-generation sequencing (NGS) of isolated T_{REG} and T_{RM} from HBV-related HCC and non-viral-related HCC identified distinct functional signatures associated with T-cell receptor signaling, T-cell co-stimulation, antigen presentation and PD-1 signaling. T_{REG} and T_{RM} from HBV-related HCC expressed more PD-1 and were functionally more suppressive and exhausted than those from non-virus-related HCC. Furthermore, immunosuppression by $PD\text{-}1^+T_{REG}$ could be reversed with anti-PD-L1 blockade. Using multiplexed tissue immunofluorescence, we further demonstrated that T_{REG} and T_{RM} contributed to overall patient survival: T_{REG} were associated with a poor prognosis and T_{RM} were associated with a good prognosis in HCC.

In conclusion, our current study provides a comprehensive immunophenotyping of tumor microenvironments in HBV-related versus non-viral-related HCCs. This improved deep understanding of the immune landscapes from HCC with different etiologies will likely contribute to better disease management for HCC, especially in the context of immunotherapy.

TH148. Amplification of Neoantigen-specific Antitumor Immunity using a Long-lasting IL-2 Fusion Protein

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Tumor-specific T cells are central mediators of antitumor immunity; however, approaches to harness their potential have had limited benefit due to the low frequency of these cells in patients. IL-2 is an agent to boost tumor-specific T cells, but its efficacy has been minimal due to toxicity and off-target effects. We developed a long-lasting IL-2/CD25 fusion protein that exhibits more favorable pharmacokinetics than IL-2. Here we show that, a single administration of high dose IL-2/CD25 enhances the *in vivo* expansion of melanoma-specific $CD8^+$ Pmel-1 and $CD4^+$ TRP-1 transgenic T cells after priming with cognate peptide and LPS. This expansion was accompanied by the development of a high frequency of persistent memory cells for Pmel-1 T cells. In this context, IL-2/CD25 administration delayed B16.F10 melanoma growth, extended mice survival, and supported an increased frequency of melanoma-specific T cells in the tumor. We adapted this approach to amplify endogenous polyclonal

melanoma-reactive T cells, which is more clinically relevant. Optimal expansion of these cells required a prime/boost regimen with a mixture of neoantigen peptides, Poly (I:C) and administration of IL-2/CD25. This immunization scheme delayed pre-established B16.F10 and enhanced mice survival. Characterization of the immune composition within the tumor microenvironment revealed a heightened immune response in vaccinated mice receiving IL-2/CD25, which was accompanied by increased frequencies of granzyme B- expressing T cells and decreased expression of Treg survival markers. Our data indicate the potential utility of long-acting IL-2/CD25 to amplify endogenous tumor-reactive T cell responses and overcome toxicities seen with IL-2 therapy.

TH149. A New Melanoma Vaccine Enhances Prototypic CD8+ Effector T Cells Tumor Infiltration Inhibiting Tumor Growth Even in the Absence of Anti-PD1

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Immune checkpoint blocker (ICB)-immunotherapy has shown survival benefits for cancer patients. Nevertheless, many individuals remain refractory or acquire resistance to treatment, encouraging exploration of complementary active immunotherapies. Accordingly, cancer vaccines become an attractive complementary healing alternative. Until now, experimental melanoma-based vaccines induced only weak responses, requiring the concomitant use of recombinant cytokines, proinflammatory factors or ICB to control tumor growth. Optimal delivery of tumor antigens combined with potent adjuvants seems to be crucial for vaccine effectiveness. Here, a prototype for a generic melanoma vaccine, named TRIMELVax, was tested in the weakly immunogenic B16F10 model. The vaccine is made of heat shock-treated tumor cell lysates combined with a mollusk hemocyanin as adjuvant. A human melanoma lysate (TRIMEL) contributes with danger signals that promotes antigen presentation, while murine B16F10 lysate provides melanoma-associated antigens, which were effectively cross-presented *in vivo* by conventional dendritic cells type 1 (cDC1). TRIMELVax, but not the single vaccine components, inhibited tumor growth by enhancing prototypic CD8+ effector T cells and cDC1 infiltration, and preventing accumulation of difunctional CD8+ T cells and immune suppressor cells at tumor site, even in absence of anti-PD1 concomitant treatments. The strong immunogenicity showed by TRIMELVax encourage the design of clinical studies in melanoma patients.

TH325. Blockade of PD-L1 Leads to Activation of Myeloid Inflammation in Human Cancer

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Blockade of PD-1/PD-L1 pathway can lead to tumor regression in several cancers. While PD-1/PD-L1 have different expression patterns and alternate receptors/ligands, blockade of PD-1 /PD-L1 has been studied interchangeably in the clinic, mostly in the context of T-cell stimulation. Data comparing genomic signatures of PD1 and PD-L1 blockade in humans in vivo are lacking. In order to evaluate this, we sort-purified T cells and monocytes at early timepoints from patients undergoing checkpoint blockade. In contrast to anti-PD-1 therapy, which predominantly led to gene expression changes in T cells, anti-PD-L1 therapy led to a distinct inflammatory signature in CD14+ monocytes in vivo. PD-L1 blockade also led to an increase in myeloid-derived cytokines (e.g. IL-18) not observed following PD-1 blockade. To further dissect the effect of PD-L1 blockade in early cancer, we analyzed patients enrolled in a clinical trial of atezolizumab to prevent myeloma. Atezolizumab led to rapid but transient T cell activation, as well as activation and persistence of inflammatory myeloid cells in the marrow. Blockade of PD-L1 on monocyte-derived dendritic cells (DCs) synergized with CD40L to enhance DC maturation as well as antigen specific T cell activation in vitro. These data show that PD-L1 blockade leads to distinct systemic immunologic effects compared to PD-1 blockade, particularly manifesting as myeloid activation. These findings also identify an additional underappreciated role for PD-L1 as a checkpoint for regulating antigen-presentation, which may be harnessed to improve future combination therapies.

TH332. The skin in long-term melanoma survivors maintains durable melanoma-reactive resident memory CD8 T cells

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Melanoma patients who develop immunotherapy-related adverse events often have durable responses to treatment, yet the mechanisms are not well understood. We selected four of these responders/long-term survivors who had the autoimmune adverse event vitiligo and performed single-cell RNA sequencing on $CD8^+$ T cells sorted from their matched skin, tumor, and blood. We found that tumor-infiltrating (TIL) and skin-derived CD8 T cells overlapped transcriptionally; both having transcriptional features of tissue-resident memory T cells (T_{RM}). Three distinct T_{RM}-like clusters were identified with one expressing high levels of *IFNG* and *TNF* transcripts and low levels of inhibitory checkpoint genes (T_{RM}-IFNG). To infer tumor specificity of T cells in skin and blood, TILs were used as a reference. Thirty-three expanded TCR clonotypes were present in skin and tumor and fifteen also had a counterpart in blood, indicating that circulating and T_{RM} cells can share a common clonal precursor. Triple-tissue matched clonotypes were preferentially found in the T_{RM}-IFNG cluster but were excluded

from the exhaustion cluster, suggesting that the suppressive tumor microenvironment does not alter the activation profile of T_{RM} cells. TCRβ sequencing of tumor, skin, and blood collected from seven patients longitudinally revealed markedly higher TCR clonal overlap between skin and tumor than between blood and tumor. Some clones from tumors were identified in skin several years later, confirming their long-term persistence as memory. This study illustrates the similarities between skin-resident and tumor-resident memory T-cell populations in melanoma patients and underscores the skin as a durable reservoir of melanoma-reactive T cells.

TH337. Thymic stromal lymphopoietin controls progression of colorectal cancers

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Thymic stromal lymphopoietin (TSLP) is well known to be secreted from intestinal epithelial cells and as a modulator of homeostasis and diseases including various cancers. However, the role of TSLP in colorectal cancers has not been fully understood. Using the Azoxymethane/Dextran Sodium Sulfate-treated mouse model of colitis-associated cancer, we found that both TSLP and TSLP receptors (TSLPR) were expressed on colorectal cancer cells. Deletion of TSLPR on colorectal cancer cells partially impaired in the generation and growth of colorectal tumors. Of note, we also found that a subset of Treg cells expressed high levels of the TSLPR and Treg-specific deletion of TSLPR led to fewer colorectal tumors in compared to that of control mice. Further analysis showed that Treg-specific deletion of TSLPR led to decreased numbers of Treg cells in the tumors. These data implied that TSLP controls Treg cell homeostasis and colorectal cancer cell survival to progress colorectal cancers. Importantly, TSLP blockade following the tumor initiation partially ameliorated to a marked reduction in tumor growth. Finally, TSLP receptor-expressing Treg cells was enriched in colorectal cancers in patients. Collectively, our study suggests that TSLP plays an important role in the progression of colorectal cancers in human and mouse. Thus, TSLP is a potential therapeutic target for colorectal cancers.

TH350. The CXCR3 Chemokine System Determines the Function of Stem-like CD8+ T cells and Allows for Treatment Stratification in Solid Tumors

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The chemokine receptor CXCR3 plays a major role for the tumor infiltration of CD8+ T cells, yet it remains unclear how the human spliced variants of CXCR3 (CXCR3V: A/B/alt) shape anti-tumor function. We identify the highest expression of all CXCR3V in human memory stem T cells (TSCM) among CD8+ T cell subsets, including the CXCL11-specific variant CXCR3alt. TSCM display a stem-like, early-differentiated T cell state that is crucial for long-lasting immune responses. To elucidate the functional role of the CXCR3 chemokine system for CD8+ T cells, we conducted migration and

expansion assays applying the CXCR3-ligands CXCL9/10/11. Intriguingly, stem-like CD8+ TSCM show the highest chemotactic response to CXCL11 >9 >10 among CD8+ T cells. Surprisingly, CXCL11 was the only CXCR3-ligand supporting effector function of TSCM in a tumor-specific expansion system. This sheds light on a potential stimulatory CXCL11-CXCR3V axis relevant for anti-tumor immunity. Human systems are required for investigation of the CXCR3V, as mice lack the spliced states of CXCR3. Exemplary, we conducted a study on a cohort of muscle-invasive bladder cancer (MIBC) patients. In pre-treatment MIBC, we found a synergistic predictive capacity of the CXCL11-CXCR3alt pathway that completely segregated responder from non-responder (chemotherapy) patients (n=20). Validation was performed in an independent TCGA cohort comparing chemotherapy treated (n=68) versus chemo-naïve MIBC patients (n=292). In summary, we identified a novel stimulatory pathway based on the spliced state of CXCR3 (CXCR3alt) expressed in CD8+ TSCM responding to CXCL11. The CXCR3alt-CXCL11 chemokine system may be of general use for treatment stratification in solid tumors.

TH352. Bridging MOA-based ADCC Reporter Bioassay with an Improved PBMC-based ADCC Cytotoxicity Assay for Immunotherapy mAb Development

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Measurement of antibody-dependent cellular cytotoxicity (ADCC) is critical for understanding antibody Fc effector functions during monoclonal antibody development. Classic ADCC assays measure the short-term cytotoxicity of target cells, typically pre-loaded with radioactive or fluorescent dyes, after exposure to antibody and primary PBMCs or NK cells. These assays are widely used in antibody discovery and characterization during early drug development. However, the use of primary effector cells makes them vulnerable to high assay variability and therefore not suitable for use in a quality-controlled environment during product manufacture and development.

Previously, we developed an ADCC reporter bioassay using engineered ADCC effector cells and demonstrated its specificity and ability to measure an ADCC mechanism of action. The assay is prequalified according to ICH guidelines; demonstrates precision, accuracy, linearity, and robustness; and is suitable for product release and stability studies in a quality-controlled environment. In order to enable bridging studies comparing PBMC-based ADCC cytotoxicity assays and ADCC reporter bioassays, we recently developed an improved ADCC cytotoxicity assay using PBMCs and engineered HiBiT target cells. When HiBiT-expressing target cells are incubated with an antibody and PBMCs, the target cells are lysed and release HiBiT peptides, which then bind to LgBiT in the detection reagent to form a functional Nano-luciferase to generate luminescence. The assay is simple, homogenous, highly sensitive, and gives a robust assay window. It shows antibody potency comparable with the ADCC reporter bioassay in ADCC bridging studies.

TH353. Investigation of the Predictive Utility of a Type 1 Diabetes Genetic Risk Score in Immune Checkpoint Inhibitor Induced Diabetes Mellitus

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Immune checkpoint inhibitor diabetes mellitus (CPI-DM) is a rare but morbid immune related adverse event (irAE) without known predictive biomarkers. Using a previously published type 1 diabetes (T1DM) genetic risk score (GRS) that differentiates T1DM from type 2 diabetes mellitus (T2DM) through T1DM associated single nucleotide polymorphism (SNPs) (Oram, Diabetes Care 2016), we investigated whether this GRS predicts CPI-DM. DNA samples from 15 patients with CPI-DM and 14 patients with CPI hypophysitis (CPI-HP) were genotyped on Illumina SNP arrays. The median GRS for subjects with CPI-DM (0.24) was significantly lower than the previously published GRS for T1DM (0.28, Wilcoxon Rank Sum (WRS) $p < 0.01$) and not different from the published median score for T2DM (0.235, WRS $p=0.2078$). The median GRS for CPI-DM subjects was significantly higher than the median score for CPI-HP subjects (0.21, WRS $p< 0.01$). Lower GRS for CPI-DM compared to T1DM patients could be attributable to differences in race/ethnicity and HLA. CPI-DM subjects below the median T1DM GRS lacked high risk HLAs. All three Asian CPI-DM subjects had HLAs that confer T1DM risk in Asians (DR4/DQ4), which is not accounted for in the T1DM GRS. The median GRS for the eight Caucasian Non-Hispanics was 0.25, which was not significantly different than the median scores for T1DM ($p= 0.06$) nor T2DM ($p=0.31$). These results show the limited utility of the existing T1DM GRS score in predicting development of CPI-DM, but suggests that the GRS may differentiate irAE risk especially if race/ethnicity is addressed.

TH367. Correlations of immune checkpoints and T cell exhaustion markers with disease stage and tumor budding in colorectal cancer patients

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Despite recent advances in colorectal cancer (CRC) treatment, there is a large proportion of patients showing limited responses to therapies, in particular those with advanced disease stages. Therefore, there is an urgent need to identify prognostic biomarkers and/or therapeutic targets for CRC advanced stages aiming to improve the efficacy of current treatments. We aimed to determine prognostic biomarkers in the tumor tissue and circulation of CRC patients, with a special focus on T cell exhaustion markers including immune checkpoints (ICs). We investigated correlations between the expression of several T cell exhaustion markers and clinicopathological parameters, such as disease stage and tumor budding. We found that increased mRNA levels of TIM-3, CTLA-4, TIGIT, KLRG1, TOX2, TOX3, SIRT1, Ki-67 and PRDM1 in tumor tissues were positively correlated with disease stages. Additionally, increased mRNA levels of PD-1, TIM-3, TIGIT, KLRG1, TOX4, Ki-67 and PRDM1 in tumor tissues were positively correlated with high grades of tumor budding, which is an indicator of poor prognosis and metastasis. In the circulation of CRC patients, only LAG-3 and VISTA mRNA levels showed positive correlations with disease stages. The levels of PD-1 in the tumor tissue and circulation of CRC patients were inversely correlated with staging, suggesting that targeting PD-1 in patients with advanced stages could be less effective. Collectively, these findings suggest some T cell exhaustion markers that could be as prognostic biomarkers, and potentially therapeutic targets for CRC. However, further

investigations and validations in larger cohorts of patients are required to confirm their use as biomarkers.

TH400. Investigating the Immunometabolism and Anti-tumor Properties of invariant Natural Killer T (iNKT) Cells

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Invariant natural killer T (iNKT) cells are a CD1d-restricted subset of T cells with potent innate- like anti-tumor properties. iNKT cells are primed, “poised effector” cells that could be therapeutically exploited cellular immunotherapy. However, a greater understanding of their basic cellular properties within the nutrient-poor tumor microenvironment (TME) is still needed. One major challenge faced by conventional T cell (T_{CONV})-based solid tumor immunotherapies is their ability to survive and maintain effector function long-term within the TME, competing with tumor cells for limited nutrients. While T_{CONV} metabolically reprogram upon activation to preferentially rely on glycolysis to fuel effector functions, the metabolic profile of iNKT cells at baseline, upon stimulation, and within the TME is not known. We hypothesize that iNKT cells display a memory-like metabolic program whereby they are predominantly oxidative and less reliant on glycolysis for anti-tumor functions. Our studies find that human PBMC-derived rested and stimulated iNKT cells are less reliant on glucose and glutamine to maintain effector functions *in vitro* than T_{CONV}. Transcriptional profiling of these cells for metabolic pathway genes revealed that stimulated T_{CONV} have greater upregulation of glycolysis and Myc pathway gene expression than iNKT cells, while iNKT cells display higher expression of fatty acid oxidation (FAO) genes. Using flow-based dyes, we also find differences in mitochondrial mass and membrane potential in these cell types. Further studies will elucidate the metabolic activity of iNKT cells relative to T_{CONV} and link metabolism to effector function both *in vitro* and within the TME.

TH415. Four Cases of Immune Check Point Inhibitor Diabetes, CPI-DM, after Pembrolizumab presenting as Fulminant Type 1 Diabetes with Diabetic Ketoacidosis

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Type 1 diabetes, T1DM, manifesting as Diabetic Ketoacidosis (DKA) is a rare, but severe, immune related adverse effect of programmed cell death-1, PD-1, inhibitors used in cancer immunotherapy. Their ligand, PDL-1, is expressed in the pancreatic beta-cells in T1DM. Antibodies against beta-cell antigens (Insulin, GAD65, IA-2 and Zinc transporter 8) are biomarkers for T1DM prediction. HLA DR-DQ genotypes largely exert their influence on the risk of islet autoantibodies. In the presence of a family history of T1DM, HLA DRB1*03 and HLA DRB1*04 confer a high risk for T1DM.

We report four cases of fulminant T1DM after immunotherapy with Pembrolizumab, a PD-1 inhibitor, presenting as diabetic ketoacidosis (DKA) caused by severe insulin deficiency in all four patients. Three out of four patients had normal pre-treatment glycemia before onset of DKA.

Cases:

59-year old female. Malignant Melanoma, 12 cycles. C-peptide 136. GAD65 negative.

HLA-DRB1*03. Nephew T1DM.

76-year old female. Non Small Cell Lung Cancer, NSCLC, 2 cycles. C-peptide 11. GAD65 positive. HLA-DRB1*04, maternal grandfather T1DM.

67-year old male. NSCLC, 3 cycles, C-peptide < 33, GAD65 positive, no family history of T1DM.

62-year old male with preexisting T2DM. NSCLC, 8 cycles, C-peptide 29, GAD65 positive, daughter T1DM.

The features of CPI-DM in four cases is presented. The majority have a family history of T1DM and/or are GAD65 positive. Two carry HLA DRB1*03 or DRB1*04 high risk subgroups.

A characterization of CPI-DM is needed to reduce the overall morbidity and may also lead to understanding the pathogenesis of spontaneous T1DM.

TH453. Unique Populations of PD-1, TIGIT, and Tim-3 Co-Expressing T Cells and CD56 Bright and Dim NK Cells Found in Cervical Cancer Patients

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PD-1/PD-L1 blockade has become an effective tool for treatment of different cancers. Recently, these therapies have been approved for cervical cancer. However, less than 40% of cervical cancer patients see clinical benefits from this treatment. If a patient is non-responsive to PD-1 axis blockade, it is possible that other checkpoint pathways may be implicated.

The aim of this work was to evaluate PD-1, PD-L1, TIGIT and Tim-3 expression in cervical cancer, the third most prevalent female cancer in Mexico, where this study was performed.

We found that PD-1, TIGIT and Tim-3 are over-expressed on peripheral blood $CD56^{dim}$ and $CD56^{bright}$ NK cells and T cells in cervical cancer patients compared to healthy donors. Increases in the following subpopulations were also found: $PD\text{-}1^{+}/TIGIT^{+}$ T and $CD56^{bright}$ NK cells, $PD\text{-}1^{+}/TIGIT^{+}/Tim\text{-}3^{+}$ T and $CD56^{bright}$ NK cells, $PD\text{-}1^{high}/TIGIT^{+}$ T cells and $PD\text{-}1^{+}/NKG2^{+}$ T cells. As expected, these significant increases were not observed in all patients. However, a subset had levels much higher than the healthy donor median, up to five and six and fold higher in the case of some cell populations.

When soluble PD-L1 was analyzed by ELISA, again only a subset of patients was significantly higher than the healthy median. These sPD-L1 high patients had the highest percentages of $PD\text{-}1^{+}$ T and NK cells, and were associated with positivity for the other checkpoint markers. Thus, soluble PD-L1 expression and the over-expression of multiple checkpoint markers might define an "exhausted" phenotype found in a subset of cervical cancer patients particularly amenable to new therapies.

Infectious Diseases

F215. Immunization against Pf bacteriophage prevents Pseudomonas aeruginosa wound infections

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Pseudomonas aeruginosa (Pa) is a gram-negative bacterium linked to extensive morbidity and mortality in the setting of diabetic foot wounds, pressure ulcers, and burns. Pf bacteriophage, a temperate filamentous phage that infects *Pa*, has a commensal relationship with *Pa* whereby Pf promotes increased surface adhesion and decreased motility of Pseudomonas. Pf is also taken up by immune cells and triggers type I interferon production, inhibition of tumor necrosis factor production, and ultimately the suppression of phagocytosis. It is likely through these mechanisms that Pf promotes *Pa* wound infection in mice and is associated with chronic *Pa* wound infections in humans. We identified a consensus sequence of the predominant coat protein of Pf. The infection rate in full thickness wounds is decreased in mice that are vaccinated with a peptide version of this epitope, as well as with transfer of passive humoral immunity in the form of monoclonal antibodies. Furthermore, monoclonal antibodies directed against Pf promote phagocytic engulfment of *Pa in vitro*, but this phagocytic activity is abrogated upon addition of Fc block. It is thus possible that antibody-mediated recognition of Pf phage facilitates *Pa* phagocytosis via opsonization of Pf phage adherent to type IV pili on the surface of *Pa*. In summary, we have demonstrated that a bacteriophage can be targeted to prevent *Pa* wound infections. Given filamentous bacteriophage may play a similar role in other gram-negative organisms, immunization against phage virions could be a promising strategy for the prevention of other infections.

F232. Potency and efficacy testing of a human lymphatic filariasis vaccine, rBmHAXT (LFGuardTM)

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The potency and efficacy of a GLP manufactured tag-free multivalent fusion protein vaccine, rBmHAXT (LFGuardTM) for human lymphatic filariasis (LF) was tested in Mongolian gerbils, CD1 mice and Balb/c mice. For the potency testing, gerbils were immunized with a low dose (0.5 ug), medium dose (15 ug), and high dose (40 ug) of the vaccine plus alum adsorbed GLA (AL019) as adjuvant given subcutaneously at monthly interval. The CD1 mice were immunized with a range of doses from 0.5 to 40 ug/dose. In the gerbil, 40 ug dose resulted in the maximum titer of antigen-specific IgG antibodies (1:40,000). However, in CD1 mice, 15ug was sufficient to give the maximum titer (1:20,000). Therefore, we chose 15 ug dose to evaluate the vaccine efficacy following a challenge infection in the Balb/c mouse model. All vaccinated mice had significantly ($p< 0.05$) high levels of antigen specific IgG1, IgG2a, IgG2b and IgG3 antibodies compared to control animals. Two weeks after the last dose of the vaccine, all mice were challenged with 10-15 infective third stage larvae of Brugia malayi using a micropore chamber method. Our results showed that the rBmHAXT vaccine conferred 81.8% protection in CD1 mice and 85.36% protection in Balb/c mice. Correlates of vaccine-induced protection showed an increase in the memory cells in the spleen of vaccinated mice. These findings suggest that LFGuardTM could be developed as the first human LF vaccine that could benefit about 893 million people who are at risk of acquiring the infection.

F235. Socioeconomic Position is Associated with Infection Outcome Following Iatrogenic Exposure to Hepatitis C Virus.

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Health is socially patterned - high socioeconomic position (SEP) is associated with decreased risk of mortality from cardiovascular disease, cancer and trauma, faster wound healing, and increased antibody responses to vaccines. Epidemiologically these differences have been well described. From a biological perspective however, factors that underpin increased susceptibility to premature death in low SEP groups have yet to be uncovered. We have recruited a unique pilot cohort of Irish women previously iatrogenically exposed to hepatitis C virus to determine the factors that contribute to differential health outcomes between low and high SEP. Using education status dichotomised by attendance/non-attendance at third level as a surrogate marker for SEP we found a significant association between SEP and infection outcome ($p< 0.05$; Chi-square test; n=15 per group). Low SEP had increased risk of acute and chronic HCV infection, while those from high SEP appeared to be protected, with an increased likelihood of clearing infection in an antibody independent manner. Examination of the whole blood transcriptomic profile between SEPs showed increased IL7R expression with low SEP relative to high SEP, independent of cell counts, smoking status and exercise.

No difference in transcripts of genes in the IL7R STRINGdb network at baseline were observed between SEPs. Increased IL7R expression has been implicated as a risk factor for several immune related disorders, including type 2 diabetes and multiple sclerosis. A better understanding of the interactions between IL7R and increased mortality risk in low SEP may help to identify new preventative or therapeutic clinical strategies.

F251. Conserved CSF HIV Antibody Response in Patients with Diverse Neurologic Phenotypes

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The CNS is exposed to HIV during primary infection and likely continuously during untreated chronic infection. Neurosymptomatic (NS) CSF escape is a condition in which CNS HIV infection develops despite plasma viral suppression. To assess whether NS escape might be triggered by an unidentified CNS pathogen and/or whether the CSF anti-HIV antibody repertoire might distinguish NS escape, CSF was collected from 25 HIV-infected participants, some longitudinally, with diverse neurologic phenotypes. CSF samples were incubated with a VirScan T7 bacteriophage library. Antibody-bound phage were immunoprecipitated and deep sequenced to quantify enriched viral peptides. Separately, metagenomic next-generation sequencing (mNGS) of CSF RNA was performed. mNGS was 100% concordant with HIV RNA PCR for samples with ≥ 530 viral copies (n=15) and 0% concordant from samples with ≤113 viral copies (n=8). In addition, mNGS detected the two known infections in the secondary escape patients. Additionally, the CSF anti-HIV antibody repertoire primarily enriched two distinct epitopes within the HIV envelope protein, regardless of neurologic or treatment status. These epitopes mapped to the V3 loop near the binding site for CCR5 and to the C-terminal heptad repeat domain. CSF mNGS did not identify additional infections in HIV NS escape. Preliminary VirScan data suggest that immunodominant epitopes in the CNS are highly conserved across patients, regardless of their neurologic status. Compared to anti-HIV epitopes previously described in sera, we identified CSF antibodies specific for the R306S mutation in the gp120 V3 region which has been associated with brain-derived env sequences and increased macrophage tropism.

F272. The interaction between CD43 and Mycobacterium tuberculosis chaperone Cpn60.2 results in different biological outcomes in macrophages and lymphocytes

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The lack of an effective anti-tuberculosis vaccine, along with the emergence and persistence of multidrug-resistant strains of *Mycobacterium tuberculosis (Mtb)*, makes the eradication of tuberculosis difficult. One critical piece to understand the pathogenesis and outcome of the Mtb infection is the study of the interactions between Mtb's PAMPs and their matching PRRs on immune cells. Recently, the CD43 sialomucin was reported to interact with Cpn60.2, a chaperone localized in the mycobacterial capsule.

The fact that CD43-/-macrophages produce less TNF-a in response to *M. avium* or Mtb, that Cpn60.2 interacts with CD43, facilitating the mycobacteria-cell adhesion, and that Cpn60.2 is a potent stimulator of leukocytes, led us to investigate how Cpn60.2 impacts macrophages and T cells responses.

Stimulation of human THP-1 cells or mouse BMMs with Cpn60.2 led to TNF production in a CD43-dependent manner. The expression level of CD43 negatively correlated with bacterial load, as THP-1 cells expressing lower amounts of CD43 had a higher bacterial load. Also, CD43-/- mice produced less IFN-γ and IL-17 when challenged with Mtb than WT mice, underscoring a role for CD43 and T cells in Mtb containment. Overall, Cpn60.2 lowered the expression level of CD40L and CD25 and the amounts of pro-inflammatory cytokines in CD4 and CD8 human lymphocytes following TCR-CD28 or TCR-CD43 engagement.

Thus, the Cpn60.2-CD43 interaction results in different biological outcomes in macrophages and T lymphocytes, suggesting a role for this interaction in shaping the granuloma, where the host defense elements and the pathogen confront each other.

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F316. Respiratory Syncytial Virus infection increases susceptibility to mycobacterial colonization.

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The human respiratory syncytial virus (hRSV) is the leading cause of severe lower respiratory tract infection in infants and elderly. HRSV-infection is associated with an inefficient development of adaptive immunity, which in part is driven by cytokines secreted by lung epithelial cells, increasing susceptibility to reinfection. Therefore, hRSV has gained interest as a potential primary pathogen favouring secondary opportunistic infections. Here, we evaluated whether hRSV changes the host immune response to subsequent pathogens using intranasal instillation of attenuated *Mycobacterium bovis* as a model for secondary infections. Eleven and twenty-one days-post infection we evaluated leukocyte dynamics in lungs by flow cytometry, the expression of key genes involved in immune response by quantitative PCR. Our data suggest a primary infection with hRSV significantly increases the bacterial respiratory pathology, characterized by increased infiltration of neutrophils and high counts of myeloid cells with an inflammatory macrophage profile and promotes a dormancy state of the mycobacteria in the host cell. In addition, *in vitro* experiments showed that lung epithelial cells remained infected with

hRSV despite being exposed to attenuated *M. bovis*. On the contrary, primary infection with BCG impairs hRSV infection and pulmonary pathology in mice due the overproduction of lipid droplets. Our data suggest that hRSV severely impairs the local pulmonary immune response favouring a secondary mycobacterial colonization in the lungs.

F326. Infant T cells exhibit enhanced TCR signaling and reduced self-renewal during viral respiratory infections.

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Infants are highly susceptible to recurrent respiratory infections partly due to lack of established immunological memory and protective T cell-mediated immunity. The mechanisms by which early adaptive T cell responses develop during infancy remain unclear. Here we show that T cells from both infant humans and mice exhibit enhanced T cell activation and signaling and reduced self-renewal potential. Using an *in vivo* model with co-transferred infant and adult ovalbumin-specific (OTII) CD4 T cells followed by infection with recombinant PR8-OVA influenza, we found that infant T cells exhibit increased proliferation and reduced expression of the stem-like transcription factor TCF1, compared to adult OTII cells in the lungs. *In vitro* activation further reveals that mouse infant OTII T cells show increased T cell receptor (TCR)-coupled signaling at a lower antigen dose than adult OTII T cells as assessed by enhanced upregulation of the early signaling molecules Nur77 and IRF4. Human infant lymph node T cells also recapitulate the increased TCR signaling and reduced TCF-1 expression following stimulation compared to adult counterparts. Infant T cells also have increased phosphorylation of ERK1/2 after stimulation, indicating that the enhanced TCR sensitivity of infant T cells occurs proximal to TCR triggering. Together, these results show enhanced TCR sensitivity in infant T cells govern an intrinsic mechanism that enables robust primary responses and pathogen clearance when immunological memory is absent.

F333. HIV-1 Therapy with Monoclonal Antibody Elicits HIV-1 Specific T Cell Responses in the Host

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As an alternative to standard anti-retroviral therapy (ART), anti-HIV-1 antibodies have been successful at decreasing viral loads in HIV-1 positive individuals. Through the formation of antibody–HIV-1 immune complexes, antigen presenting cells and natural killer cells are potentially activated, which would lead to cross-presentation of HIV-1 antigens to $CD8^+$ T cells or antigen dependent cellular cytotoxicity (ADCC).

To understand the effect of antibody infusion on the human host immune system, we studied peripheral blood mononuclear cells of HIV-1 positive individuals, who received 3BNC117 and/or 10-1074, two

broadly HIV-1 neutralizing antibodies. Using mass cytometry and high dimensional analysis, we identified an NK cell population as well as a CD8+ T effector memory RA^+ population that increased after antibody infusion. Mean clonality of T cell receptors increased after infusion and our network analysis using GLIPH captures a strong shared HIV-1 specific CD8+ T cell response at weeks 3-6, that is not readily seen before the infusion or at later stages. In addition, the antibody infusion enabled a more diverse CD8+ T cell response to both Gag and Nef epitopes.

Our data show that "passive" anti-HIV-1 antibody infusion in HIV-1 infected individuals affects the host NK- and HIV-1 specific $CD8^+$ T cell compartment. This finding is relevant as the synergistic effect of the antibody and host immune response could be exploited in future by combination regimens that further support the host immune response.

F336. An anti-inflammatory neutrophil subset modulate inflammation during pneumococcal pneumonia

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Neutrophils are known for their role in bacterial clearance and implication in pathogenesis of infectious diseases. However, recent reports have described that neutrophils have the ability to induce an anti-inflammatory response. Our laboratory has shown that during pneumonia caused by *Streptococcus pneumoniae*, neutrophils are able to produce IL-10 but further characterization of these neutrophils is required. In this study we have characterized neutrophils populations present during the first 48 h post-infection, providing evidence about the existence of at least two predominant populations, where the larger is the one producing IL-10. Analysis by transmission electron microscopy revealed that these two populations exhibit differences in size, granularity and chromatin condensation. Next, we performed neutrophil transfer assays to C57BL/6 wild type (WT) and $IL\text{-}10^{-/-}$ mice (the latter highly susceptible to *S. pneumoniae* infection) to determine the role of IL-10–producing neutrophils during pneumococcal pneumonia. We transferred WT and $IL\text{-}10^{-/-}$ neutrophils to each group followed by infection with *S. pneumoniae*. A 10-days survival assay was performed. Likewise, we evaluated lung infiltration of pro-inflammatory cells, as well as bacterial burden in lungs and other organs after 24 h post-infection. These results showed that transferred $IL\text{-}10^{-/-}$ mice were less susceptible to *S. pneumoniae* infection than untreated $IL\text{-}10^{-/-}$ mice. In contrast, we observed that WT mice transferred with WT neutrophils showed an increased clinical score, decreased survival and a reduced bacterial clearance. Our results strongly suggest that IL-10-producing neutrophils may modulate lung immune responses, playing a critical role during the first 48 h of *S. pneumoniae* infection.

F365. Protective Immune Response induced by Antigenic Proteins of Mycobacterium tuberculosis encapsulated in biopolymeric nanoparticles

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Mycobacterium tuberculosis (*M tb*) proteins have a significant role in the development of protective immune responses. The precise mechanism of immune modulation by biopolymeric nanoparticles remains unclear. The study focuses on whether antigenic proteins encapsulated in biopolymeric nanoparticles can induce a Th1 immune response, which plays a key role in protection against *M. tb* infection. Chitosan nanoparticles were prepared by ionic gelation method and Culture Filtrate Proteins (CFP) - CFP-10 and CFP-21 of *M. tb* were encapsulated in ChN. The binding efficiency of nanoparticles with CFP-10 and CFP-21 proteins was confirmed by UV-Spectrophotometer. The efficacy of nanoparticles-encapsulated antigenic proteins administered intraperitoneal against *M tb* aerosol infection was evaluated in Balb/c mice. Cytokine ELISA was done to assess the immune response and protection study was done by bacterial counts [CFU]. The size of the nanoparticles was measured by Dynamic Light Scattering (DLS) ~200 nm and confirmed by the TEM. The surface charge of the nanoparticles was ~41mV. The characteristic peak of the chitosan nanoparticles was observed at 1564.03cm-[1] by FT-IR Spectroscopy. The entrapment efficiency of CFP- 10 and CFP- 21 proteins was ~ 16% and 18% respectively. CFP-10 and CFP-21 proteins primed cells demonstrated a Th1 bias T cell response in an *ex vivo* assay. The results suggested that the ChN-CFP10 nanoparticles have both protective and therapeutic potential against *M tb*.

F426. An 8-gene model to predict severe dengue progression

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Of the estimated 100 million people per year who develop acute dengue fever, approximately 5-20% progress to severe dengue (SD). Supportive care reduces mortality from SD, but early detection of SD progressors remains challenging, as warning signs for SD are broad and nonspecific. To this day, there exists no prognostic assay that can accurately predict which dengue patients will progress to SD. Here, we identify and validate a host response-based transcriptional signature that predicts SD progression. We applied a modified version of our lab's multi-cohort analysis framework to 11 public datasets, encompassing 7 countries, all ages, and microarray and RNA-seq platforms. Amidst such biological and technical heterogeneity, we identified genes that were robustly associated with SD progression. To identify a parsimonious gene set, we used a greedy forward search algorithm that resulted in the 8 most highly predictive genes. We then trained a gradient boosting decision tree model using these 8 genes that classified SD progressors and non-progressors in public datasets with an area under the receiver operating characteristic curve (AUROC) of 0.91. Finally, we validated the 8-gene model in an independent Colombia cohort consisting of 376 prospectively enrolled dengue patients (all ages). In this cohort the 8-gene model predicted SD progression with an AUROC of 0.83. Relative to clinical warning signs, the 8-gene model improved specificity for SD progressors by nearly 4-fold, indicating its utility for triage of dengue patients, particularly in high-burden and limited-resource settings.

TH23. "Effect of the Inhibition of p-AKT and p-ERK1/2 on the Expression of Hormonal Receptors in THP-1 Monocytes and Macrophages Infected with T. gondii and Stimulated with 17-β Estradiol"

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Background: *Toxoplasma gondii* (*T. gondii*) is a parasite common in pregnancy. Monocytes and macrophages are the main immunologic barrier, because they produce proinflammatory cytokines, reducing the infection susceptibility. This outcome is highly regulated by signaling pathways such as MAPK (ERK1/2) and PI3K (AKT), important in cell growth and proliferation. 17β-estradiol and *T. gondii* activate these pathways, and thus, modulate the expression of hormonal receptors (ERα, ERβ, GPER, and PRLR) and proinflammatory cytokines.

Aim: To evaluate the effect of the inhibition of signaling molecules p-AKT and p-ERK1/2 on the expression of hormonal receptors in THP-1 monocytes and macrophages infected with *T. gondii* and stimulated with 17β-estradiol.

Methods: THP-1 monocytes were cultured on RPMI medium supplemented with BFS 10% and antibiotics. PMA was used to differenciate them into macrophages. Cells were kept at 37°C having 5 % of CO2. Inhibition of AKT and ERK1/2 was performed with Akt inhibitor V Triciribine (20 μM) and PD098059 (50 μM) respectively. Stimuli were performed with 17β-estradiol (10 nM), *T. gondii* (20,000 tachyzoítes) and mixed with both conditions for 48 hours. Proteins were extracted and quantified, and Western Blot assays were performed.

Results: After 60 minutes, the inhibition of pAKT and pERK1/2 was complete. ERα and ERβ expression decreased after *T. gondii* infection on monocytes and macrophages when p-AKT or p-ERK1/2 were inhibited. Finally, the expression of PRLR increased by mixed stimulus on monocytes after inhibition of both signaling pathways.

Conclusion: Inhibition of p-AKT and p-ERK1/2 modifies the expression of hormonal receptors in monocytes and macrophages.

TH43. Analyzing CD4+ T cell responses to M. tuberculosis using GLIPH2 and whole genome antigen screening

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Analyzing the ab T cell responses to pathogens in human beings is a daunting task, especially because of the diversity of HLA alleles (>20,000), and the complexity of many pathogen genomes which can encompass thousands of proteins. Here we have adopted a two-pronged strategy to both broadly analyze the CD4+ T cell response to Mycobacterium tuberculosis (Mtb), and to develop a way to identify specific CD4+ T cell antigens that emerge from the analysis. The first prong of this strategy is to

sequence and analyze the T cell receptor beta (TCRb) chains from 58 latently infected individuals using an improved version of our GLIPH algorithm (GLIPH2) on 19,044 unique TCRs. This clusters TCRs based on shared motifs in the complementarity-determining region 3 (CDR3) and other similarities. The second prong of our approach is the development of an efficient method to screen the entire genome of Mtb for CD4+T cell epitopes of interest. This involves expressing subpools of 3,724 open reading frames (ORFs) in Mtb and then having these proteins processed and presented by artificial APCs (aAPC) transfected with the indicated class II HLA genes, which are then surveyed by reporter T cells transfected with the TCR heterodimers of interest. We show that this works efficiently and successfully in discover new T cell specificities. This coupling of TCR repertoire analysis together with a whole pathogen genome screen to discover novel peptide-MHC specificities represents a powerful new strategy that can be applied to any well characterized pathogen response, in any organism.

TH102. Trained innate immunity in vaccine protection against intrarectal AIDS viral transmission

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Although anti-Envelope antibodies have been viewed as the primary means of protection against AIDS viral acquisition, we have found in two of AIDS vaccine studies in rhesus macaques that protection against intrarectal viral transmission can be achieved in the absence of anti-envelope antibodies, but rather mediated by myeloid cells through trained innate immunity. In the first study, a vaccine involving an envelope construct and modified vaccinia Ankara encoding SIV antigens was used mucosally, with TLR ligands, IL-15, and mutant E coli labile toxin as adjuvants. Protection was achieved (VE= 44%) even though no anti-envelope antibodies could be detected in the blood or colorectal mucosa. T cells were induced but did not correlate. Rather, protection correlated with CD14+ monocytes. More IL-6, TNFa and Mip1a was produced from vaccinated animals when re-exposed to virus than those from control animals. In the second study, animals were vaccinated with a live attenuated SHIV, whose envelope does not crossreact with SIV envelope. No antibodies to SIV envelope could be detected, yet the animals had reduced risk of rectal transmission of SIVmac251 (VE = 81%). T cells were induced but did not correlate with protection. Moreover, CD8 T cell depletion of protected animals did not lead to re-challenged SIV infections. Transcriptomic studies suggested gene expression changes were induced in the myeloid cells from the protected animals. We conclude that trained innate immunity was involved in protection against AIDS virus transmission. This provides new approaches to AIDS vaccine development.

TH103. Evaluation of Immune Response to Hepatitis B Vaccine After Primary Immunization in Sudanese Children Aged 10 Years and less

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Background: Although the long-term protection against HBV infection depends on the persistence of anti-HBs antibodies and strong immunological memory, the anti-HBs antibodies level declines after

vaccination.
Objectives: The study conducted to determine the effectiveness of the Hepatitis B vaccine and to evaluate its long-term protection in Sudanese children aged ≤10 after primary vaccination.
Methods: This is cross-sectional hospital-based study included 225 subjects aged 10 years and less. All subjects received the three-dose schedule of hepatitis B vaccine as part of their primary vaccination. Blood samples collected after approved consent and the sera tested for anti- HBs level using automated immunoassay technique.
Results: All children with the age group of less than one year have a seroprotective rate of anti-HBs (>10 IU/L). Compared to 63.3%, 50%, 72.7%, 50%, 43.8% and 33.3% of participants aged 5, 6, 7, 8, 9 and 10 years, respectively. The Geometric Mean Titer (GMTs) of anti-HBs was 419.7 IU/L, 110. 2 IU/L, 71.07 IU/L, 99.2 IU/L, 53.7 IU/L, 48.7 IU/L, and 17.7 IU/L % of participants aged < 1, 5, 6, 7, 8, 9 and 10 year, respectively. In children with the age groups of 5-10 years, 51.5% of them have anti-HBs level >10 IU/L compared to 48.5% have < 10 IU/L. 17.6% of the participants have an undetectable level of anti-HBs (< 2 IU/L). **Conclusion:** Hepatitis B vaccine in Sudan succeeded in provoking the immune response against HBV for up to 10 years after primary vaccination. The anti-HBs level and its GMTs decreased with age.

TH138. KIRs modulate the immune response to P. falciparum malaria

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KIRs (killer immunoglobulin like receptors) are a family of 14 transmembrane receptors that can be either inhibitory or stimulatory. Nearly all of the known KIR ligands are different MHC-I alleles and groups of alleles. KIR and compound KIR/HLA genotypes have been associated with outcomes from HIV, HCV, HPV and other infectious diseases. However, very few studies have assessed the effect of KIR and their HLA ligands on *P. falciparum* malaria. In a large Ugandan cohort, we analyzed the effect of KIR/HLA genotypes on parasite prevalence and clinical malaria outcomes. We found that more inhibitory KIR/HLA pairs led to higher parasite prevalence but did not affect other outcomes. To understand the effect of KIR at the cellular level, we have studied KIR expression and function on both NK cells, the canonical KIR expressing cell type, and gamma delta T cells which also express KIR and have been show to play an important role in the immune response to *P. falciparum* infection. Specifically, we demonstrated that subsets of both NK cells and gamma delta T cells that are more likely to express KIR increase with repeated *P. falciparum* episodes. Further, we found that KIR are able to modulate the response of a subset of gamma delta T cells to their *P. falciparum* ligand. Taken together our results show a role for KIRs in modulating the immune response to *P. falciparum* both via NK cells and via gamma delta T cells.

TH155. CD137 costimulation enhances the antiviral activity of Vγ9Vδ2-T cells against influenza virus

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Influenza epidemics and pandemics are constant threats to global public health. Although strategies including vaccine and antiviral drugs have achieved great advance to control influenza virus infection, the efficacy of these strategies is limited by the highly frequent mutations in the virus and the emergence of drug-resistance strains. Our previous study indicated that boosting immunity of human Vγ9Vδ2-T cells by phosphoantigen pamidronate could be a therapeutic strategy to treat seasonal and avian influenza virus infections. However, one notable drawback of γδ-T cell-based immunotherapy is the rapid exhaustion of proliferation and effector responses due to the repeated treatments with phosphoantigens. Here, we found that the expression of CD137 is inducible in Vγ9Vδ2-T cells following antigenic stimulation. $CD137^+$ Vγ9Vδ2-T cells displayed more potent antiviral activity against influenza virus than their $CD137^-$ counterpart in vitro and $Rag2^{-/-}\gamma c^{-/-}$ mice. We further demonstrated CD137 costimulation was essential for Vγ9Vδ2-T cells activation, proliferation, survival and effector functions. In humanized mice reconstituted with human peripheral mononuclear cells, CD137 costimulation by recombinant human CD137L protein boosted the therapeutic effects of pamidronate against Influenza virus. Our study provides a novel strategy by targeting CD137 to improve the efficacy of gammadelta-T cell-based immunotherapy

TH158. Chlamydia trachomatis drives immunosuppression in macrophages which further mediates T cell inactivation causing persistent infection

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Chlamydia trachomatis repeated/latent infection is responsible for ectopic pregnancies, abortions, pelvic inflammatory disorders and infertility. IFNγ from T cells depletes IDO and activates $CD8^+$T cell to clear *Ct*. However, low IFNγ causes persistent infection. Infected epithelial cells skewed macrophages(mø) to alternative activated mø(AA-mø) with increased *CD163* and *arginase* levels and decreased *iNOS* levels. Infected macrophages express high CD206 and decreased MHC-II indicating polarisation to M2. On co-culture of infected mø with naïve T cells, T cells secreted IFN-γ, IL-4, IL-10 and TGFβ in various levels. IFN-γ mediated persistent infection in mø secreted increased IL-10 and differentiated T cells to high IL-4 and IL-10 producing cells. These mø expresses decreased CD40 and increased PDL-1. AA-mø on infection displayed higher bacterial load, increased IL-10 secretion, expresses high *PDI* and *Ephrin B2* and differentiated T cells to Th2 and Treg. T cell cytokines: IFNγ decreases the bacterial burden; IL-4 did not show any change and IL-10 increased bacterial burden. Acute *Ct* infection of BALB/c mice elicits Th1 response and INFγ secretion in cervical lavage. While, repeated *Ct* infection show Th2 and Treg. Further T cells express high *PD-1*, *Tim3* and *CTLA4* indicating T cell inactivation mechanisms behind decreased T cell proliferation. $CD8^+$T cells from chronic infected animals show decreased *Granzyme B* and *perforin* expression. Chronic and latent infected mice also show decreased $INF\text{-}\gamma^+CD4^+$ and $INF\text{-}\gamma^+CD8^+$ T cells compared to acute infection. In conclusion, *Chlamydia* polarises macrophages to M2 mediating immunosuppression thus decreasing T cell activation resulting in incomplete clearance leading to persistent infection.

TH165. Multi-omic profiling of influenza-specific adaptive immune responses at single cell resolution

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The immune system recognizes and eliminates non-self threats through a complex and layered network of both innate and adaptive immune cells. Robust characterization of this response and discovery of novel cell types and antigen-specific populations has proven challenging to perform in a high throughput fashion due to sample availability, the need to enrich for multiple cellular populations, and the single-modal nature of flow cytometry, CyTOF, and similar assays. Here, we apply multi-modal droplet-based single cell technologies to pre- and post-vaccination T cells, B cells, and peripheral blood mononuclear cells from influenza vaccines. Using this framework, we identify vaccine-specific T and B cell responses, show proof-of-principle for *in silico* HLA typing via single cell RNA sequencing, and implement for the first time a single B cell clonotyping algorithm.

By linking gene expression, cell surface protein expression, genotype, and antigen-specificity to each single cell, we describe a snapshot of the complete circulating immune response to influenza vaccination at single cell resolution. Single cell lineage analysis revealed post-vaccination expansion and class-switching of multiple B cell clonotypes, and parallel expansion of influenza-specific T cell clonotypes. We also observed clonotype-specific gene expression profiles that differed between antigen species (Epstein-Barr virus, cytomegalovirus, and influenza). We anticipate that these technologies will comprise a robust and cost-effective multi-omic platform for studying immunology at single cell resolution.

TH171. Pf Bacteriophage Alters Immune Profile in Epithelial Cells and Sputum from Patients with Cystic Fibrosis

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Background: Bacteriophage therapy is re-emerging as a strategy to address raising antibiotic resistance. However, the effect bacteriophage could have on the human host is not well understood. We have demonstrated that in cystic fibrosis (CF) co-infection with *Pseudomonas aeruginosa (Pa)* and Pf bacteriophage has effects on clinical outcomes. Here we sought to profile the inflammatory response to Pf bacteriophage in the CF lung.
Methods: We collected sputum from CF patients infected with Pa, 40 also infected with Pf phage and 40 without. Cytokine profiling of the samples was performed by Luminex bead-assay. To validate findings *in vitro*, nasal epithelial cells from a CF patient were cultured at air liquid interface and exposed to 4 conditions: PBS, LPS, Pf phage and Pf phage + LPS, and compared to healthy control cells

exposed to the same conditions. Fluid samples from cell culture were assayed for cytokine production. **Results:** Compared to patients without Pf, sputum from patients with Pf had elevated levels of IL12-p70 (p=0.087) and decreased levels of CXCL5 (p = 0.068), RESISTIN (p = 0.046) and TRAIL (p = 0.025), by stratified non-parametric rank test. Cell culture revealed drastically different responses to both Pf phage and LPS in CF vs non-CF cells. Specifically, in CF cells there was elevation of CXCL5 and GMCSF in the Pf and LPS condition.
Conclusion: Pf phage affects inflammatory responses in the CF lung. The potential immune effects of phage need to be further investigated.

TH205. Cetylpyridinium Chloride (CPC) Blocks Herpes Simplex Virus Replication in Gingival Fibroblasts by Interfering with NF-κB Signaling Early After Infection

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Herpes simplex viruses (HSVs) are highly prevalent worldwide and infections are lifelong. Individuals with clinical symptoms elicited by HSV infection may suffer, among others, from occasional or recurrent herpetic lesions in the orofacial and genital areas, as well as herpetic gingivostomatitis. The antiviral acyclovir, which inhibits the viral DNA polymerase is commonly used for topical treatment of HSV-associated pathology. However, it is generally poorly effective in treating lesions, as it only reduces in 1-2 days the duration of skin lesions from a total of approximately 6-7 days. Cetylpyridinium chloride (CPC) is a quaternary ammonium compound with bactericidal properties that is frequently found in mouthwashes, deodorants, aphthae-treating formulations and oral tablets. Previous reports indicate that CPC negatively modulates NF-κB activity. Because HSV infection is modulated by NF-κB, we sought to assess whether CPC has antiviral effects against HSV-1 and HSV-2. We found that a short exposure to CPC after HSV entry into human gingival fibroblasts significantly limited viral replication in these cells by impairing viral gene expression. Interestingly, our results suggest that CPC blocks HSV replication by inhibiting the expression of immediate early HSV genes, such as *ICP0* by interfering with the translocation of NF-κB into the nucleus of HSV-infected cells. Taken together, these findings suggest that formulations containing CPC may help limit HSV replication and consequently reduce viral shedding.

TH366. Inhibiting PD-1 restores protective T cell response and facilitates bacillary clearance: Impact on modulation of the Efflux pump expression of Mycobacterium tuberculosis

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Purpose: Previously, we have shown the critical role of regulatory T (Treg) cells and PD-1 pathway in causing suppressed state of T cell response against *Mycobacterium tuberculosis* (*M.tb*) among TB patients. In this study, we attempted to understand the status of host immune response among the multi-drug resistant (MDR) and Drug Sensitive (DS) TB patients. We also checked the contribution of PD-1 pathway on poly-functional T cells (PFTs) response, critical for protective immunity in TB.

Methods: Polychromatic flowcytometry based immunological assays were performedfor immune response profiling. PD1 blocking experiments were performed in mice infected with *Mtb.*

Results: We observed rescue of PFTs in TB patients by blocking PD-1. Blocking PD-1 pathway in *in vitro* monocyte derived macrophage (MDM) model and *in vivo* among *Mtb* infected mice demonstrated restoration of PFTs and reduction in bacillary load. Furthermore, we observed modulation of efflux pump of *M.tb* by pro-inflammatory (IFN-γ, TNF-α) and anti-inflammatory cytokines (IL-10 and TGF-β) in *in vitro* MDM model. Moreover, PD-1 inhibition also enhanced the effector T cell response to candidate vaccine-ID-93 *in vitro*.

Conclusions: Our results demonstrate elicitation of weaker T cell response in DR TB and rescuing immune response improves the efficacy of anti-tubercular therapy in TB patients. Additionally, we demonstrate that immune rescue by PD-1 inhibition improves the efficacy of chemotherapy as well as therapeutic vaccination in TB. We conclude that blocking PD-1 may be a novel strategy for immunotherapy and therapeutic vaccination in TB.

TH413. A blood-based flow cytometry assay for the evaluation of Immune Response in pulmonary and extra-pulmonary tuberculosis

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Tuberculosis (TB) remains a leading cause of death worldwide from an infectious diseases category, causing 1.5 million deaths annually. Currently, there are several diagnostic methods available such as microscopy, culture, PCR-based methods and ELISA, each having their own limitations. Hence, new diagnostic tools that are sensitive and provide accurate detection of pulmonary tuberculosis (PTB), Extra pulmonary tuberculosis (EPTB) and pediatric TB, using a sputum independent specimen, is an unmet need.

Here, we report a rapid, sputum-independent flow cytometry-based assay for the evaluation of immune response to PTB and EPTB in adult and immunocompromised suspects. This assay evaluates immune response to *Mycobacterium tuberculosis* (MTB) by identifying the TB specific effector T cell responses. This assay incorporates phenotypic T cell characterization; identification of production of Interferon gamma (IFN-γ) on effector T cells to investigate and differentiate between tuberculosis disease and infection and/or no disease state.

This study reports the outcome of a prospective, multi-site study where the flow cytometry-based assay was evaluated against multiple reference methods (recommended by WHO). The results and interpretation from the reference methods (Likely or Unlikely TB) were used to generate a receiver operating characteristic curve for the flow cytometry test. The Overall the AUC was at 0.831 with a lower limit of 0.7550 and upper limit of 0.9070.

Inflammation and Host Defense

F5. Early IFNb Secretion Determines Variability in Downstream IL-12p70 Responses upon TLR4 Activation in Health and Disease

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IL-12p70 is a heterodimeric pro-inflammatory cytokine which regulates both innate and adaptive immunity and has a central role in Th1 responses. Therefore, IL-12p70 holds great potential as a target for new diagnostic and therapeutic strategies. Despite its essential role in immunity we have observed large variation in IL-12p70 responses in 1,000 healthy donors of the *Milieu Interieur* cohort. Furthermore this perturbed response was observed at significantly higher frequencies in patients chronically infected with tuberculosis (TB) or chronic hepatitis C (HCV). We have applied a systems biological approach in the *Milieu Interieur* cohort to dissect this perturbed pathway in healthy donors, and potentially provide new insights into disease pathogenesis. Following whole blood LPS stimulation using a highly standardized approach from 1,000 healthy donors of the cohort, 28% of these donors failed to secrete IL-12p70. Having identified by flow cytometry that monocytes and dendritic cells are the main IL-12p70 producers in whole

blood, we confirmed that cellular differences were not responsible for the observed variability in healthy donors. In contrast, genetic analysis of the healthy population cohort revealed a novel association with IL-12p70 levels. Gene set enrichment analysis identified significant differences in both type I and type II IFN responses, with a detailed kinetic analysis mapping this upstream to variable IFNb responses, acting through IFNAR1 signaling. These results will allow us to investigate the intracellular mechanisms by blocking specific pathways and to potentially restore them in chronically infected HCV or TB patients.

F218. Distinct Inflammatory Ageing Process in People Living with HIV

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People living with HIV (PLWHIV) develop frailty, an ageing-related syndrome that increases the likelihood of poor healthcare outcomes, at an at an earlier age than HIV-negative individuals. Chronic inflammation may drive the frailty phenotype in PLWHIV, known as inflammageing. We hypothesise that ageing PLWHIV develop distinct immune cell populations and inflammatory behaviour compared to HIV-negative individuals, causing earlier development of the inflammageing phenotype. We recruited 8 young HIV- (median 25y), 7 older HIV- (median 53.5y), 7 young PLWHIV (median 34y) and 10 older PLWHIV (median 54.5y). We assessed frailty across multiple domains. We measured lymphoid and myeloid immune cell populations via flow cytometry and production of the inflammatory cytokine interleukin-6 by ELISA in peripheral blood mononuclear cells (PBMCs)

Ageing in both PLWHIV and HIV-negative populations is associated with longer timed get up and go (TUG) (PLWHIV p= 0.0015; HIV-negative p= 0.007) and lower MOCA (PLWHIV p= 0.0117; HIV-negative p= 0.0357). Ageing in PLWHIV is also associated with increased systemic C-reactive protein levels (CRP; p= 0.0137). Ageing in HIV-negative individuals is associated with an increased proportion of CD4+ T cells and reduced proportion of CD8+ T cells. Both older groups had lower proportion of CD16+ cells in CD45+ HLA DR+ monocyte population compared to their younger counterparts. PBMCs from older PLWHIV had higher IL-6 production following exposure to the inflammatory agonist LPS versus older HIV-negative individuals (p= 0.0413).

These results suggest a distinct inflammageing phenotype in ageing PLWHIV versus ageing HIV-negative individuals.

F234. A valine to leucine substitution in IFNAR1 alters IFN-signaling and is associated with increased susceptibility to acute hepatitis C virus infection in humans.

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Type I interferons (IFN) are important in host protection against viral infection. Here we investigated the impact of a genetic variant (rs2257167) in the IFN receptor subunit, IFNAR1, on outcome following iatrogenic exposure to highly-infectious batches of hepatitis C virus (HCV) contaminated anti-D immunoglobulin. A gene candidate approach revealed a valine to leucine substitution in IFNAR1 at position 141 was associated with increased susceptibility to acute HCV infection, while the wildtype G allele was associated with innate protection against HCV ($p< 0.05$; Fisher's exact test; n=204). 3D visualisation of the IFNAR1 using PyMOL localised the SNP to the SD2 domain and may impact IFN binding and signaling. Blood from participants (n=30) was stimulated with a panel of anti-viral agonists (polyI:C, R848, ODN2216, and IFNα) using the TruCulture system. Transcriptomic analysis revealed differential responsiveness to stimulation associated with the rs2257167 polymorphism in IFNAR1. PolyI:C stimulation showed the most striking differences between genotypes; GG individuals showed increased IFN-stimulated gene regulation compared to those with a C allele. Proteomics on supernatants from stimulated TruCulture tubes using Luminex and SIMOA revealed enhanced cytokine responses in the GG group compared to those with a C allele following PolyI:C stimulation only. The differential responses between donors suggests a difference in regulation of pathogen recognition receptor (PRR) signaling by IFNAR1 driven by the presence/absence of the C allele. Future work will shed light on how genetic variation in IFNAR1 shapes PRR responses and contributes to differential outcome during viral outbreak.

F248. iNKT Cell Inhibitory Receptor Signatures Are Altered with ART-Suppressed HIV Infection and Aging and Tracks with Plasma Markers of Inflammation and Coagulation

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Invariant NKT (iNKT) cells are a unique population of innate-like T cells that exert inflammatory or immunoregulatory functional profiles and can impact chronic disease onset and progression. iNKT cells are reported to be functionally impaired in viremic HIV infection and with normal aging. Virally-suppressed HIV-infected individuals have earlier onset and increased risk of age-associated diseases which are linked with elevated plasma inflammatory cytokine levels. The potential additive impact of virally-suppressed HIV infection and older age on circulating iNKT cells is unknown. Here, we measured iNKT surface phenotypes and compared with 16 plasma markers of inflammation, coagulation, and intestinal permeability from PBMC samples of our HIV and Aging cohort which includes ART-suppressed HIV+ individuals and matched uninfected controls divided into younger (≤35yo) and older (≥50yo) groups. iNKT frequencies were similar between the four groups; however, differences in inhibitory receptor (IR) expression were found, including higher percentages of PD-1+ cells in older compared with younger uninfected subjects and the highest levels of multi-IR+ cells found in the HIV+ older group. Also, positive correlations were found between iNKT IR signatures and plasma analytes associated with age-related co-morbidities. Taken together, these data indicate that age and aviremic HIV infection impact the circulating iNKT cells and that in turn, this unique T cell population may contribute to the 'inflamm-aging' found with both aviremic HIV and normal aging. Further investigation into the diversity and regulation of iNKT subsets may reveal novel therapeutic targets to reduce the inflammation found with HIV infection, aging, and other inflammatory conditions.

F262. Interleukin-21 Production Marks an Activated T Helper Population in Cardiovascular Disease

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T cells play a critical role in cardiovascular diseases including hypertension. Interleukin-21 (IL-21) has been shown to be necessary in initiating immune activation in mouse models of hypertension and sustaining end-organ damage. CD4+ T cell production of IL-21 is also elevated in hypertensive humans and correlates with systolic blood pressure. The aim of this study is to define the specific CD4+ T cell populations that produce IL-21 in hypertension to identify novel cellular targets. At baseline, VFP+ CD4+ T cells express significantly higher levels of the follicle homing receptor CXCR5 and higher levels of the T cell activation markers LAG3 and PD-1 than VFP- CD4+ T cells. VFP+ cells are high in levels of the activation marker CD44, in agreement with previous studies. To model hypertension, 10-12 week-old mice heterozygous VFP reporter mice were infused with the vasopressor angiotensin II or a vehicle control for four weeks. There was no change in numbers of circulating or splenic VFP+ CD4 cells in animals infused with angiotensin II compared to controls. Splenic CD4 cells did significantly increase their expression of LAG3 in hypertensive mice compared to controls, which occurred in both VFP+ and VFP- T cells. Further studies will examine the ability of VFP+ CD4+ T cells to provide help to B lymphocytes and other T cell subsets as well as evaluate any changes in VFP+ T cells in the vasculature during experimental hypertension.

F274. In vitro activation of macrophages by a MHC class II-restricted Trichomonas vaginalis TvZIP8-derived synthetic peptide

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Macrophages play an important role in the inflammatory response towards pathogens and their effector functions depend of the mode of activation which is mediated by recognition of pathogen-associated molecular patterns, as peptides. *Trichomonas vaginalis* provokes an inflammatory response in the host in which macrophages are the first line of defense. The aim of this study was to analyze the effect of a specific peptide derived from the transporter TvZIP8 of *T. vaginalis* on activation of macrophages. An immunoinformatics approach was applied to screen potential murine MHC class II-restricted peptides from TvZIP8 that can activate macrophages. Based on binding scores one of them, denominated TvZIP8-pep, was selected for further analysis. *In vitro* stimulation with synthetic TvZIP8-pep triggered on murine macrophages the NO and H_2O_2 production and an overexpression of *iNOS* and *NOX-2* genes. Also, a significant increase of pro-inflammatory cytokines: IL-1β, IL-6, and TNF-α, as well as, overexpression of the *TLR4*, *MyD88* and *NF-κB* genes and NF-κB activation were observed on macrophages after stimulation with TvZIP8-pep *in vitro*. Moreover, higher levels of IFN-γ were detected in co-cultures using CD4+ T cells with TvZIP8-pep-stimulated macrophages. These results supports the potential of TvZIP8 as a promising antigen to stimulates a specific macrophage response.

F312. The Unfolded Protein Response programs T cell effector differentiation

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Generation of protective adaptive immunity against pathogens and cancer requires signals from the T-cell receptor (TCR), costimulation, and cytokines to induce transcriptional, epigenetic, and metabolic programs that instruct naïve T-cells differentiation into various effector and memory subsets. Despite its intricate role in sensing and integrating cellular metabolism and bioenergetics in differentiation cells, the specific contribution of the Unfolded Protein Response (UPR) to T-cell differentiation is unclear. The UPR consists of three arms – IRE1α, PERK, and ATF6 – activated in response to unfolded proteins in the endoplasmic reticulum (ER) to restore ER proteostasis. The most-studied and evolutionarily conserved enzyme IRE1α acquires endoribonuclease activity upon activation, splicing cytoplasmic XBP1 mRNA to encode a potent transcription factor that promotes expression of protein chaperones. PERK-induced ATF4 and activated ATF6 induce gene networks that enhance ER protein folding and alleviate proteotoxic stress. Whether UPR enzymes act independently or interdependently during immunity is also not known. We have found that all three UPR arms are induced during T-cell activation *in vitro* and *in vivo* in a manner dependent on CD28 co-stimulation. Strikingly, while mice with T-cell-specific deficiency of individual UPR enzymes mounted normal responses to lymphocytic choriomeningitis virus (LCMV) Armstrong infection, mice containing T-cells devoid of all three UPR enzymes markedly failed to generate antiviral T-cell responses. Subsequent RNA-seq validation revealed an exhaustion signature in UPR-deficient T-cells previously only seen in wild-type chronic LCMV infection. These studies demonstrate a previously unappreciated redundant and essential role of the UPR during antiviral T-cell responses.

F382. Identification of a Kupffer cell subset capable of reverting the T cell dysfunction induced by hepatocellular priming

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Kupffer cells (KCs) are highly abundant, intravascular, liver-resident macrophages long known for their scavenger and phagocytic functions. KCs are also able to present antigens to $CD8^+$ T cells and promote either T cell tolerance or full effector differentiation, but the mechanisms underlying these discrepant outcomes are poorly understood. Here, we used a mouse model of hepatitis B virus (HBV) infection – where HBV-specific naïve $CD8^+$ T cells recognizing hepatocellular antigens are driven into a state of immune dysfunction – to identify a subset of KCs (referred to as KC2) that improves the antiviral function of T cells upon IL-2 administration. Mechanistically, KC2 were found to be both enriched in the IL-2 sensing machinery and poised to cross-present hepatocellular antigens in response

to this cytokine. Removing MHC-I from all KCs – including KC2 – as well as selectively depleting KC2 impaired the capacity of IL-2 to revert the T cell dysfunction induced by intrahepatic priming. Together, these findings indicate that, by sensing IL-2 and cross-presenting hepatocellular antigens, KC2 overcome the tolerogenic potential of the hepatic microenvironment and suggest new strategies for boosting T cell immunity in the liver.

TH68. Effect of Honey and Cigarette Smoke on Lung Inflammation

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Introduction: Honey is used as a traditional medicine for colds, skin inflammation but not edible. Cigarette smoke (CS) is a significant risk factor in the pathogenesis of pulmonary diseases. Alveolar macrophages (AM) are known to play an essential role in lung defense. However, it is unclear that CS and honey effect on lung inflammation. In this study, we investigated whether CS and honey affect lung inflammation by Lipopolysaccharide (LPS). **Materials & Methods:** Mice were exposed to CS for 10 days. Mice inhaled 60μg of LPS by intranasal administration (CS-LPS). Mice inhaled 600μg of Japanese honey. Mice not exposed to CS inhaled LPS (NS-LPS). Alveolar macrophage (AM) and neutrophil (Neu) were obtained by broncho-alveolar lavage (BAL). Expression of TLR4, CD14 surface antigen and production of reactive oxygen species (ROS) were analyzed by FACS. Chemotactic activity was measured by EZ-TAXIScan. Cytokines and NF-κB mRNA expression were assayed by RT-PCR. **Results:** Neu counts were significantly increased with LPS inhalation. Expression of TLR4 in neutrophils and AM was significantly decreased in CS-LPS. ROS production of Neu was significantly increased in CS-LPS. IL-1β, TNF-α, CXCL1 and NF-κB mRNA expressions of Neu were not different between NS-LPS and CS-LPS. Honey inhibited chemotactic activity and ROS production of Neu. Honey inhibited IL-1β and CXCL1 mRNA expression of AM. Honey also inhibited infiltration of neutrophils to the lung. **Conclusions:** These results suggest that inhibition by CS may increase pulmonary infection. Honey may indicate anti-inflammatory activity via the suppression of infiltration of neutrophils.

TH114. The role of alpha beta T cells in the progression of coronary artery disease

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Background: Atherosclerosis-related diseases are a leading cause of death worldwide. Patients with atherosclerosis are at risk of developing heart attack and stroke, which may result in death. While previous murine studies have revealed how immune response regulation in T cells may play a crucial role in the promoting development of atherosclerosis, studies in humans are limited.

Objective: To genotype the CDR3 region of the T cell receptor (TCR), which is the primary binding site for the MHC-antigen complex, in T cells isolated from coronary atherosclerotic plaque.

Methods: Plaques were digested in single cell suspension and sorted into CD4+ and CD8+ T-cells in 96-well plates using fluorescence-activated cell sorting (FACS). From there, RT-PCR was performed on the RNA from the cells and two sets of nested PCR were performed specific for the α or β regions of the TCR. The DNA was barcoded then sequenced using Illumina MiSeq. This data was then used for clonality and CDR3 motif analysis.

Findings: FACS data analysis revealed significant presence of αβ T cells in the plaque of our cohort. T cells were clonally expanded in plaque, suggesting an antigen specific response. GLIPH analysis of the sequencing data revealed that patients had T cells with a shared TCR β CDR3 motif, suggesting a common antigen.

Conclusion: Our data suggests there is an antigen specific response by T cells in coronary atherosclerotic plaque. Further investigation is required to identify potential disease-causing antigens that activate these T cells.

TH122. Increased atrial mast cell numbers are associated with reduced fibrosis: a potential role for IL-33

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Mast cells (MCs) are immune sentinels that respond to infection and tissue damage via selective production of granule products, lipid mediators and cytokines. IL-33 is a dual function cytokine that can be actively secreted or released via damage to structural cells. Human MCs respond to IL-33 via production of multiple mediators. After tissue injury in the heart, improper resolution of inflammation leads to fibrosis and heart failure. The role of cardiac MCs in regulating myocardial inflammation remains to be defined but current evidence suggests that IL-33 may limit fibrosis. We hypothesized that MCs promote resolution of cardiac inflammation and reduced fibrosis, potentially through IL-33. Atrial appendage from cardiac surgery patients (n=112) was assessed for collagen and MC density by histology and MC molecular signatures by droplet digital PCR. Clinical variables and outcomes were also followed. Human MC responses to IL-33 were examined *in vitro*. MCs were present in human atrial tissue at varying densities determined by histology and droplet digital PCR. Patients with high MC density had less fibrosis (p=0.0073) and lower severity of heart failure classification than patients with low MC content (p=0.0066). IL-33 activated human MCs produced the anti-fibrotic cytokine VEGF-A *in vitro*, among others. Expression of *IL33* and *VEGFA* genes was positively correlated in patient atrial tissue, suggesting this relationship is active in this cohort. These findings suggest a key role for mast cells and IL-33 in regulating fibrosis following tissue damage which may be of importance in other mast cell rich tissues.

TH150. Neurotoxic Effect Of Ipomoea aquatica On The Central Nervous System Of Albino Mice.

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Background: Water spinach (*Ipomoea aquatica*) is most commonly grown in East and Southeast Asia. It is an indigenous green leafy vegetable and herbaceous aquatic or semi-aquatic perennial plant is found abundantly in coastal areas of Bangladesh. Extract of *Ipomoea aquatica* exhibited high antioxidant properties with hydrophilic-oxygen radical absorbance capacity and 2,2-diphenyl-1-picrylhydrazyl scavenging activity being 341.92 ± 1.32 and 37.67 ± 2.63 µmol Trolox equivalent/gram of dry weight respectively. The extract also showed to be neurotoxic to the animal especially on Central Nervous System.

Methods: Albino mice of the five experimental groups (AF1, AF2, AF3, AF4 and AF5) were treated for 10 days, with an aqueous fraction of *Ipomoea aqautica* leaf extract diluted in normal saline water in order to obtain doses of 5g/kg, 10g/kg, and 20g/kg and 25g/kg.

Results: Neurotoxic behaviors were observed in the experimental groups such as the behavioral response of movement, unconsciousness, loss of appetite resulting in weight loss at the high dose concentration, symmetrical ataxia as compared to control. Dose-dependent cellular vacuolation, a centric nucleus, edema, spongy degeneration, cellular necrosis, lipodosis, and hyper chromatic nuclei were observed. Histopathological lesions such as degeneration neurons are more prevalent in the higher dose groups AF3 and AF4 as compared to AF1, AF2 groups.

Conclusions: The results suggested that *Ipomoea* leaf extract is a potent neurotoxic agent affecting the nervous system of albino mouse brain. So, this biological agent has drastic effect on brain tissue. Research implicates that *Ipomoea aquatica* may be considered as bio pesticide for future use.

TH210. Regulation of monocyte function by Epstein-Barr Virus Interleukin-10 (vIL-10)

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Epstein-Barr Virus (EBV) is a herpes virus that maintains latency in infected B cells and shows intermittent reactivation. EBV is associated with certain malignancies and autoimmune diseases such as systemic lupus erythematosus (SLE). EBV encodes homologs of several human proteins to overcome host immune response and establish latency. One such lytic-phase protein, EBV interleukin 10 (vIL-10), is a homolog of human IL-10 (hIL-10). We observe increased vIL-10 in SLE patient sera, however, the impact of vIL-10 on innate immune cells is unclear. vIL-10 induces significantly lower STAT3 phosphorylation in human monocytes compared to hIL-10, and is less efficient in downregulating inflammatory genes. hIL-10, but not vIL-10, downregulated the gene expression of IL-10

receptor alpha subunit (IL-10R1). We observe a similar pattern with IL-10R1 protein expression, suggesting reduced receptor internalization and/or degradation with vIL-10 stimulation. vIL-10 signals through IL-10R1, since a neutralizing antibody to IL-10R1 inhibited STAT3 phosphorylation induced by either hIL-10 or vIL-10. Interestingly, vIL-10 inhibited upregulation of suppressors of inflammatory response by hIL-10. hIL-10 stimulation significantly increases IL-10R1 co-localization with late endosome marker, LAMP2, suggesting internalization and degradation of IL-10R1. However, vIL-10 stimulation increases IL-10R1 co-localization with Rab11, a marker for recycling endosomes. Our data suggest that vIL-10 alters IL-10R1 internalization and downstream signaling processes. The changes in receptor processing may allow vIL-10 to compete with hIL-10 and inhibit downregulation of inflammatory responses. We propose that a resulting increase in inflammation may exacerbate autoimmune responses in chronic inflammatory diseases such as SLE.

TH217. Emergence of an Inflammageing Phenotype in Older People Living with HIV, and the Possible Role of HLA-DR+ CD16+ Monocytes

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People living with HIV (PLWHIV) develop frailty and ageing-associated comorbidities at an earlier age than HIV-negative individuals. Two inter-linked cellular processes frequently altered by ageing and potentially associated with the frailty phenotype are autophagy, which involved removal and/or recycling of damaged or unnecessary organelles, and inflammation (or “inflammageing”). We hypothesise that there is altered inflammation and autophagy in immune cells of PLWHIV, leading to the frailty phenotype developing. We recruited 10 older PLWHIV (median 54.5y) and 6 older HIV-negative individuals (median 53.5y). We performed a frailty assessment across multiple domains. We assessed mitochondrial ROS and autophagic flux (through LC3-II co-localisation) in lymphoid and myeloid cells via flow cytometry.

Older PLWHIV have increased C-reactive protein (CRP; p= 0.028) and urea (p= 0.042). Older PLWHIV have reduced number of CD4+ T cells, and increased number of CD8+ T cells and increased proportion of CD8+ T cells in the CD45+CD3+ subset. There is no difference in mitochondrial ROS or autophagic flux amongst lymphoid populations. HLA DR+ CD16+ non-classical monocytes displayed increased mitochondrial ROS production (p= 0.0496) but no significant change in autophagic flux (p= 0.08) in older PLWHIV versus older HIV-negative individuals. These results show the emergence of an inflammageing phenotype in older PLWHIV, as well as distinct immune populations changes in these groups. They also identify the HLA-DR+ CD16+ monocyte population as a potential contributor to dysregulated inflammation and autophagy, leading to the increased frailty in this cohort.

TH391. Investigating the Role of Cystathionine Gamma-Lyase in the Myeloid Cell-Driven Pathogenic Immune Response to H. pylori

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Activated gastric macrophages (Gmacs) play a key role in inflammation during *Helicobacter pylori* (*Hp*)infection. We recently determined that macrophages infected with *Hp* express cystathionine g-lyase (CTH), an enzyme of the reverse transsulfuration pathway (RTP). The RTP is dependent on the metabolism of S-adenosylmethionine (SAM), a substrate also required for polyamine synthesis. Our Aim was to determine if CTH has an *in vivo* role in gastric inflammation during *Hp* infection and if this is related to alterations in polyamine metabolism. CTH expression was increased and localized to gastric $CD68^+$ cells in *Hp*-infected WT mice compared to uninfected. In $Cth^{-/-}$ vs WT mice, gastritis severity was reduced in both an acute and chronic *Hp* infection model, 4- and 8-week PMSS1 and 16-week SS1 respectively. The upregulation of genes encoding pro-inflammatory markers and the Mreg/Treg cytokine IL-10 that occurred in infected WT mice was completely eliminated in $Cth^{-/-}$ mice. Consistent with the attenuated immune response, *Hp* colonization was increased in $Cth^{-/-}$ mice with both strains of *Hp*. We found that CTH activity in Gmacs alters polyamine synthesis, as deletion of *Cth* resulted in an increased ratio of spermidine to putrescine in infected mice. Additionally, Gmacs from $Cth^{-/-}$ mice had significantly altered expression of metabolic and immune pathways as determined by RNAseq, and modulated biochemical pathways involving polyamines, methionine, and cysteine, in gastric tissue as determined by a metabolomics approach. Thus, CTH represents a new therapeutic target to limit gastritis and potentially, the related risk for carcinogenesis.

Inflammatory Diseases

F47. CyTOF Analysis of Human Colon and Blood Reveals Distinct Immune Signatures of Ulcerative Colitis and Crohn's Disease

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Background: The mucosal dysregulation underlying inflammatory bowel disease (IBD), as well as inflammatory signatures differentiating ulcerative colitis (UC) and Crohn's disease (CD), are not fully understood. Considering many IBD patients have disease refractory to available therapies, defining

these signatures will aid in identifying novel therapeutic targets, improving diagnostic clarity, and potentially tailoring individual treatment approaches.

Methods: We applied mass cytometry (CyTOF) to colonic mucosa (n=87) and peripheral blood (n=85) of patients with active(*a*) or inactive(*i*) UC/CD and non-IBD controls. CyTOF analysis was supplemented with scRNAseq, flow cytometry, RNA *in-situ* hybridization, and application of Random Forest predictive modeling.

Results: Novel IBD immune signatures were identified. Active IBD mucosa was characterized by increased abundance of (1) HLA-DR$^+$CD38$^+$ T cells, including pro-inflammatory cytokine-expressing memory Tregs; (2) CXCR3$^+$FoxP3$^+$ plasmablasts; (3) IL-1b$^+$ macrophages/monocytes. Signatures specific to UC*a* mucosa included (1) expansion of IL-17A^{++} MAIT cells, IL-17A$^+$ Tregs, and decrease in IFNg$^+$TNFa$^+$ T cells; (2) expansion of HLA-DR$^+$CD56$^+$ granulocytes; (3) reduction in ILC3s. Signatures specific to CD*a* mucosa included expansion of (1) IL-1b$^+$HLA-DR$^+$CD38$^+$ T cells; (2) IL-1b$^+$TNFa$^+$IFNg$^+$ naïve B cells (3) IL-1b$^+$ DCs/pDCs. In the periphery, CD*a* differed from UC*a* by increased (1) IL-1b$^+$ Tregs; (2) IL-1b$^+$ DCs/pDCs; (3) IL-1b$^+$ monocytes; and (4) decreased group 1 ILCs. Random Forest modeling highlighted these findings and additional signatures differentiating subject groups in mucosa and periphery.

Conclusions: Our results demonstrate the strength of single-cell technology in identifying disease-specific immune signatures in human colonic tissue and periphery that can be harnessed for targeted therapeutics and personalized medicine in IBD.

F173. Inhibition of CD38 ecto-hydrolase activity with a biepitopic first-in-class antibody that lacks effector function: Potential applications in diseases related to aging and metabolism

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CD38 is a multifunctional ectoenzyme expressed on immune cells that hydrolyzes nicotinamide adenine dinucleotide (NAD+) and its precursors nicotinamide mononucleotide (NMN) and nicotinamide riboside, thus exhausting this essential co-factor from the fixed cellular pool. NAD+ depletion negatively affects cellular health, longevity and repair of DNA damage by reducing sirtuin (SIRT) and poly (ADP-ribose) polymerase (PARP) activities respectively, both requiring NAD+ as a co-substrate. Inhibiting CD38 hydrolase activity without depleting CD38 expressing cells, would provide benefit by increasing NAD+ pool in cells.

We developed TNB-738, a human biepitopic antibody that consists of two CD38 specific heavy chains paired using knob-in-hole technology. This bivalent antibody is a potent inhibitor of CD38 enzyme activity. A silenced IgG4 Fc to prevent effector functions is further engineered to prevent arm exchange

with other IgG4 antibodies. In-vitro treatment of CD38 positive cell lines resulted in markedly increased intracellular NAD+ levels and higher intracellular SIRT1 and PARP activities. Synergistic effects were observed in combination with supplemented NMN. Upon binding to CD38-positive cells, the antibody is not internalized; doesn't activate T cells; doesn't result in antibody dependent cellular cytotoxicity or apoptosis. In addition, an equivalent antibody against mouse CD38 (Ab68) was generated to study CD38 enzyme inhibition in murine models of human diseases.

In summary, these antibodies TNB-738 and Ab68 are first-in-class potent enzyme blockers that will elucidate the role of NAD metabolism in inflammatory and metabolic disorders. Teneobio has initiated development-related activities of its anti-human CD38 for the treatment of metabolic and inflammatory diseases related to aging.

F180. The Effect of Obesity on the Expression and Secretion of Pro-Inflammatory Pain Mediators from Synovial Fibroblasts in Patients with Osteoarthritis

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Osteoarthritis (OA) the most common cause of disability in older adults, often co-exists with obesity. Previous research has established a link between an increase in adipose tissue (AT) and a more inflammatory OA phenotype, but the exact mechanisms for this remain poorly understood. AT secretes inflammatory cytokines known as adipokines, which are well known regulators of metabolic homeostasis This study aimed to investigate the link between adipokines and inflammatory OA phenotypes in obese patients. We studied the effects of five adipokines, leptin, visfatin, adiponectin, resistin and IL-1β, on the expression/secretion of the pro-inflammatory pain mediators, IL-6, IL-8, CGRP, PTGS2 and βNGF, by synovial fibroblasts (SF) from patients with OA. Confluent primary osteoarthritic SF were cultured in the presence of adipokines. Real-time quantitative polymerase chain reaction and an enzyme-linked immunosorbent assay were used to quantify pro-inflammatory pain mediator expression and secretion, respectively. IL-6 production increased following simulation by visfatin, IL-1β, adiponectin and resistin ($p< 0.05$), but not leptin. No change in IL-6 secretion over time (6, 72, 96 hours) was observed following visfatin. There was an increase in IL-8, CGRP and PTGS2 expression following visfatin and IL-1β, compared to unstimulated controls, although this did not reach statistical significance. IL-1β increased PTGS2 expression ($p< 0.05$), whereas adiponectin did not. Visfatin increased βNGF expression ($p< 0.05$), whereas IL-1β had no effect. No change in βNGF secretion over time (6, 72, 96 hours) was observed following visfatin. These findings support an aetiological role for adipokines in the more inflammatory OA phenotype of obese patients.

F183. PF-06480605 Anti-TL1A Antibody in Treatment of Ulcerative Colitis: From Bench to Bedside

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Tumor necrosis factor-like ligand 1A (TL1A), a product of the *TNFSF15* gene, binds to the death receptor 3 (DR3) and enhances secretion of multiple proinflammatory cytokines, including IFN-γ, IL-2, and IL-13. Studies have shown genetic polymorphisms within, or near, the *TNFSF15* gene that alter susceptibility to inflammatory bowel disease (IBD). As TL1A expression is up-regulated in diseased bowel tissues, neutralizing monoclonal antibodies specific for TL1A have been suggested as targets in the treatment of IBD.

PF-06480605, is a first-in-class, fully human IgG1 mAb, which neutralizes TL1A with high specificity. PF-06480605 reduced production of IFN-γ, IL-2, IL-13, IL-4, IL-5, IL-17, and TNF-α while enhancing IL-10 secretion in an in vitro whole blood assay using blood from controls and IBD patients stimulated with immune complexes, IL-12/IL-18, and anti-CD3-Ab, suggesting a mechanism of action that would be beneficial in the treatment of IBD.

Following a Phase 1 clinical trial, the safety, tolerability and efficacy of PF-06480605 for the treatment of moderate-to-severe ulcerative colitis (UC) was evaluated in a Phase 2a, open-label, multicenter, single-arm study (NCT02840721) with endoscopic improvement (EI) at Week 14 as the primary endpoint. Study participants were 56.0% male, had mean age of 40.0 years and 72.0% had prior experience of anti-TNF inhibitors. PF-06480605 exhibited an acceptable safety and tolerability profile and at Week 14, statistically significant efficacy was observed with an estimated 38.2% EI out of 45 evaluable participants.

These experimental findings and clinical studies warrant further, continued evaluation of PF-06480605 in multicenter, randomized and controlled trials.

F194. Mapping the transcriptional programmes regulated by canonical cytokines in human colonic organoids provides key functional and clinical insights into inflammatory bowel disease

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Immune- epithelial interactions shape the intestinal barrier responses to environmental triggers and are central in our current understanding of inflammatory bowel disease (IBD) pathogenesis. Cytokines have been shown to play an integral part in this interaction, with some considered pro-inflammatory (e.g. IFNγ, TNFα) and others protective (e.g. IL22).
We set out to define and compare the transcriptional programmes regulated by the canonical cytokines IFNγ (TH1), IL13 (TH2), IL17A(TH17), IL22 (TH22) and TNFα, as a pro-inflammatory control, in human colonic organoids and associate them to disease phenotypes and therapeutic trajectories in IBD. Whole transcriptome profiling of cytokine treated human colonoids (n=4) was performed using the Illumina platform. Pathway analysis revealed a large functional overlap between IFNγ, IL22 and TNFα transcriptional programmes with key pathogenic pathways upregulated by all three cytokines. Interrogation of whole biopsy transcriptomic profiles from IBD patients and healthy controls (own cohort and reposited datasets: GSE59071, GSE16879, GSE23597, GSE109142, n=369) with gene set variation analysis revealed the simultaneous activation of multiple cytokine regulated transcriptional modules in both ulcerative colitis (UC) and colonic Crohn's disease. Intriguingly, patients with the same inflammatory activity had a gradient of activated cytokine regulated modules. Those with ≥2 modules activated had a higher risk of non-response to anti-TNFα therapy. Our findings provide novel insights into the human gut immune-epithelial interactome and pave the way for a more granular immunophenotyping of IBD. The simultaneous activation of multiple canonical cytokine regulated transcriptional programmes may explain the low response rates seen with current therapeutic strategies targeting single cytokines.

F220. T cell responses to proteoglycan aggrecan peptides in knee OA patients

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Knee osteoarthritis is accompanied by inflammation and infiltration of immune cells eg. T cells and macrophages within the joint-surrounding tissues, such as, the infrapatellar fat pad and synovial linings. Many studies suggest the role of an antigen-driven response in osteoarthritic patients, eg. the presence of an oligoclonal T cell pattern and Ig-producing B cells in the synovium. Previous studies have shown that OA patients were responsive to proteoglycan aggrecan peptides especially the p16-31 and p263-

280 peptide fragment. Here, we show that healthy individuals were capable of eliciting T cell responses to the p16-31 and p263-280 peptide fragments, suggesting primed T cells specific to these peptides. Interestingly, these T cells produced the pro-inflammatory cytokine, IL-6. T cells within the infrapatellar fat pad also elicited responses towards both peptide fragments. We also investigated the cytokines that were released from T cell responses in response to these peptide stimulations. In the synovial fluid of knee OA patients, many cytotoxic mediators, including IL-6, were detected at high levels.

F222. Is there evidence for antigen driven expansion of synovial CD8+ T cells in psoriatic arthritis?

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Background:

CD8+ T-cells and the IL-17/IL-23 pathway are implicated in the pathogenesis of psoriatic arthritis (PsA). We hypothesised that the synovial IL-17+ CD8+ TCRβ repertoire contains clonal expansions suggestive of antigen-driven expansion.

Methods:

TCRβ sequencing was performed on memory (CD45RA-CD27+/- and CD45RA+CD27-), IL-17+ and IL-17-IFNγ+ CD8+ T-cells from synovial fluid (n=4) and paired memory CD8+ T-cells from blood (n=2) from patients with PsA.

Results:

Whilst the TCRβ repertoire of all CD8+ T-cell populations was polyclonal (mean clonality scores 0.09-0.11), the repertoire of synovial memory CD8+ T-cells was very different from paired blood (mean Morisita Index 0.2). Several clones were significantly expanded in synovial fluid compared to blood (mean 32 clones/patient). In contrast, the TCRβ repertoires of synovial IL-17+ and IL-17-IFNγ+ CD8+ T-cells were similar (mean Morisita Index 0.6), suggesting that synovial CD8+ T-cells exhibit plasticity in terms of cytokine expression.

Grouping of Lymphocyte Interactions by Paratope Hotspots (GLIPH) analysis, which groups T-cells based on CDR3β similarity and putative shared specificity, identified 20 groups of synovial memory CD8+ T-cell clones that contained clones from all four patients, suggesting a common antigen(s).

Conclusion:

The synovial CD8+ T-cell repertoire in PsA is different from blood and shows some commonalities across patients, suggesting antigen-driven recruitment/expansion of synovial CD8+ T-cells. Current work is aimed at comparing the CD8+ TCRβ repertoire in skin with synovial tissue and the CD8+ TCRβ repertoire across multiple joints from the same patient.

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F230. Evaluating the Synergistic Therapeutic Potential of Filarial Cystatin and Macrophage Migration Inhibitory Factor-2 on DSS-Induced Colitis in Mice

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Previous studies from our laboratory demonstrated the therapeutic potential of *Brugia malayi* cystatin (r*Bma*Cys) and *Wuchereria bancrofti* macrophage migration inhibitory factor-2 (r*Wba*MIF-2) in ameliorating the clinical symptoms and pathology of colitis in a mouse model. In this study, we evaluated the synergistic therapeutic potential of a combination of r*Bma*Cys (25µg) and r*Wba*MIF-2 (25µg) in suppressing colitis symptoms and pathology following dextran sulfate sodium (DSS)-administration. Our results showed that combination therapy resulted in reduction in the bodyweight loss (6.48%); reduced disease activity index (2.8 ± 0.91); preservation of the colon length (6.9 ± 0.31 cm), and reduced histopathological changes in the colon tissue. However, these findings were not significantly different compared to r*Bma*Cys treatment (2.88%; 2.3 ± 1.49; 7.3 ± 0.47 cm respectively). Similarly, immunohistochemistry studies showed decreased infiltration of $LY6G^+$ cells, $HMGB1^+$ cells, and MPO^+ cells, and decreased frequency of $F4/80^+$PerC macrophages and MPO activity (28.02 ± 11.08 U/g) along with increased numbers of $GATA6^+$ cells in the colon tissue following combination therapy similar to the r*Bma*Cys treatment. mRNA levels of IL-10 and NOS-2/Arg-1 ratio were increased 0.02 fold and 1.5 fold respectively in the colon tissue following combination therapy, whereas, levels of TNF-α was decreased 10 fold compared to the untreated group. These findings show that combination therapy with r*Bma*Cys and r*Wba*MIF-2has no added advantage compared to monotherapy. Studies also suggest that the r*Bma*Cys and r*Wba*MIF-2 probably induce their anti-inflammatory effect through pathways that potentially interfere with each other's function.

F260. Proteomic signature of bacterial-mediated modulation of Th2 differentiation in atopic dermatitis

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A wide range of targeted therapies for Th2-mediated diseases is under development. Th2 cell differentiation is a complex mechanism involving multiple cellular pathways. Gaps exist in our understanding of the molecular effects of novel therapies on human Th2 immune responses. We

employed a proteomic approach to probe our *in vitro* model of Th2 cell differentiation and tested a bacterial-derived inhibitor. This model employs human PBMCs and atopic dermatitis molecular factors. We quantified 1790 proteins and key cellular pathways using Orbitrap mass spectrometer.
Exposure to atopic dermatitis molecular factors TSLP and SEB induces Th2 memory cell differentiation in our model. We found that, relative to control, Th2 differentiation induces significant changes in multiple cellular pathways; namely, cellular response to external stimuli ($p < 5.55 \times 10^{-15}$), metabolism of proteins ($p < 1.75 \times 10^{-11}$), metabolism of RNA ($p < 1.75 \times 10^{-11}$), programmed cell death ($p < 1.68 \times 10^{-9}$) and vesicle-mediated transport ($p < 1.88 \times 10^{-8}$), as shown using the clustering engine database *Reactome*. A detailed analysis of this proteome data revealed activation of IL-13, IL-12 and IL-1 pathways under Th2 differentiating conditions. Within these pathways, we identified the proteins SERPINB2 and STAT3 to be significantly modulated following exposure to PGN-SAndi. The upregulation of STAT3 was confirmed using flow cytometry and ImageStream, with enhanced expression of pSTAT3 and nuclear colocalization following PGN-SAndi.
This discovery phase proteome study represents a proof of concept that integrates proteome data as a powerful tool to identify candidate pathways in targeted Th2 drug development.

F305. Clinical use of PSGL-1 agonist antibody in inflammatory diseases

Shih-Yao Lin
AltruBio

Title: Clinical use of PSGL-1 agonist antibody in inflammatory diseases

PSGL-1 is well known adhesion molecule that participates the migration of certain immune cells during inflammatory conditions. About a decade ago we discovered that PSGL-1 indeed also plays a key role in negatively regulating late-stage or chronic activated T cells. This novel function has been confirmed by many other scientists using PSGL-1 knockout mice. For instance, more than 8 days post infection, normal wild type but not PSGL-1 knockout mice can efficiently eliminate majority of those virus-specific T cells further indicating the important role of PSGL-1 in maintaining T cell homeostasis of removing unwanted activated T cells.

We have been testing a humanized PSGL-1 agonist antibody, which does not influence the migration of immune cells, in various inflammatory diseases in clinics. Based on our clinic experiences with more than 180 subjects from different clinical trials in US, unlike many other immuno-regulatory antibodies, PSGL-1 agonist antibody does not influence the protective immune responses at all. Superior safety profiles and compelling efficacy have been observed. In the current presentation we like to share a few compelling results of using this novel first-in-class agonist antibody in controlling psoriasis, psoriatic arthritis and steroid refractory aGvHD. The potential therapeutic use of PSGL-1 agonist antibody for many T cell-mediated inflammatory diseases will be discussed.

F310. Diffuse Lung Disease Emerging during Cytokine Inhibitor Treatment of Systemic Juvenile Idiopathic Arthritis (sJIA)

Vivian Saper[1], Gail Deutsch[2], R. Paul Guillerman[3], Bernice Kwong[1], Karthik Jagadeesh[1], Johannes Birgmeier[1], Gaungbo Chen[1], Purvesh Khatri[1], Elizabeth Mellins[4] and the CARRA Registry Investigators[5]

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Background: Systemic juvenile idiopathic arthritis (sJIA) is a rare, chronic inflammatory disease of unknown etiology. While pleuritis is a well-known manifestation, a novel and often fatal diffuse lung disease (DLD) has recently emerged. In 2019, we reported on distinctive features of this DLD in sJIA patients treated with cytokine inhibitors of interleukin (IL)-1 or IL-6. Here, we extend our findings with additional cases and new analyses.

Methods: Multi-center, international retrospective series of 55 cases of DLD developing in sJIA during anti-IL-1/IL-6 treatment. 48 physicians provided case details. Childhood Arthritis and Rheumatology Research Alliance registry investigators provided control data.

Results: Risk factors include trisomy 21 (13%;7/55) and younger median age at sJIA onset (2.5yrs) compared to controls (n=417) without DLD (0.2%,5.2yrs). Pathology in 87% (20/23) of lung biopsies was a spectrum of pulmonary alveolar proteinosis/endogenous lipoid pneumonia. Chest CT patterns were variable and atypical for this pathology. Whole-exome sequencing (20/55) did not identify novel defects or likely causal variants. Macrophage activation syndrome during anti-IL-1/IL-6 treatment was noted in 59% (32/55) vs published rate of < 2% without known DLD. Other unusual findings included acute digital clubbing 76% (42/55), evidence of drug-related eosinophilia with systemic symptoms (DReSS) 45%(25/55), and anaphylaxis to tocilizumab (IL-6 inhibitor) 46% (17/37). Fatality rate is 29% (16/5) with median follow-up of 2.0 years.

Conclusions: A rare, life-threatening DLD in sJIA is defined by a constellation of unusual clinical characteristics occurring in association with anti-IL-1/IL-6 treatment. In some instances, drug hypersensitivity appears related. Causality and specific mechanisms are unknown.

F379. 'Pathogenic' T Cell Responses in the Development of Systemic Sclerosis and Scleroderma

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There is increasing evidence that pathogenic T cells that reside in the tissue play an important role in chronic inflammatory diseases such as fibrosis. Understanding the molecular pathways regulating tissue-resident T cell responses could lead to improved targeted therapies with increased therapeutic index resulting in durable clinical remission. To study pathogenic T cell responses in the skin, we studied a mouse graft versus host disease model of systemic sclerosis based on differences in the

minor histocompatibility loci. We observed a significant increase in ear swelling in the diseased mouse at week 4. This correlated with a significant increase in the number of ICOS+PD-1+ T cells within the diseased ear and concomitant increase in the expression of a number of genes encoding markers of immune cell regulation and migration, notably PD-L1, CXCR5 and CXCL13, and VCAM-1. Following the peak of inflammation at week 4, we observed an increase in collagen deposition in the diseased ear at week 6 that confirmed the development of fibrosis in the ear skin. To further understand the mechanisms that regulate T-cell:fibroblast interactions in mediating fibrosis, we utilized an *in vitro* fibroblast:T-cell co-culture system. Activated T blasts or simply the addition of activated T cell supernatant was able to significantly increase the expression of PD-L1 on the surface of fibroblasts. Future studies include dissecting out the role of circulating versus tissue-resident T cells, PD-1/PD-L1 interaction, and the CXCR5:CXCL13 axis in regulating T cell mediated fibrosis in the skin.

F394. Integration of Heterogeneous Molecular and Clinical Data Identifies an Association Between Decreased Colectomy Rates and Atorvastatin Exposure in Ulcerative Colitis Patients: A Retrospective Cohort Study

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Ulcerative colitis (UC) is a chronic inflammatory disorder of the gastrointestinal tract with limited effective therapeutic options for long-term treatment and disease maintenance. We hypothesized that multi-cohort analysis of independent cohorts, which represent real-world heterogeneity of UC patients, would identify a robust transcriptomic signature to improve repurposing of FDA-approved. We performed a multi-cohort analysis of transcriptome profiles of 272 colon biopsies across 11 publicly-available datasets to identify a robust UC disease gene signature. We compared the gene signature with *in-vitro* transcriptomic profiles induced by 781 FDA-approved drugs. We used a retrospective cohort study design modeled after a target trial to evaluate the protective effect of predicted drugs on colectomy risk in patients with UC from two independent cohorts, the Stanford Research Repository (STARR) database and Optum Clinformatics DataMart. The *in vitro* transcriptome profile of atorvastatin treatment had the highest inverse-correlation with the UC disease gene signature among any non-oncolytic FDA-approved therapy. In both the STARR cohort (n=827) and Optum cohort (n=7821), we found that atorvastatin use was significantly associated with a decreased risk of colectomy compared to patients who were prescribed a comparator drug (STARR: HR=0.47, p=0.03; Optum: HR=0.66, p=0.03). These findings suggest that atorvastatin might serve as a novel therapeutic option for ameliorating disease in patients with UC. Importantly, we provide a systematic framework for integrating publicly available heterogeneous molecular data with similarly heterogeneous clinical data at a large scale to repurpose existing FDA-approved drugs for a wide range of human diseases.

F427. Stimulation of Whole Blood from Rheumatoid Arthritis Samples with Lipopolysaccharide (LPS) and R848 Results in Altered Expression Patterns in the Toll Receptor Pathway

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The challenge of identifying biomarkers for rheumatoid arthritis (RA) that can be utilized to assess disease severity, predict response to therapy, and/or monitor therapy effectiveness may be overcome by investigating immune function via targeted stimulation. TruCulture®, a whole blood collection and culture system, was used to collect and stimulate whole blood from healthy donors and rheumatoid arthritis subjects at baseline and 3 months post-treatment with methotrexate. After 24 hours incubation at 37ºC, the cell layer was analyzed for differential gene expression changes upon stimulation with Null, TNFa, LPS, R848, and FSL-1 at the cellular level were analyzed using the NanoString® nCounter® Human Autoimmune Profiling Panel. The Autoimmune Profiling Panel uses direct, digital detection of 770 mRNA transcripts using nCounter chemistry with oligonucleotide-linked fluorescent barcodes to profile 35 annotated pathways, and processes involved in autoimmune disease and chronic inflammatory disorders. Differential analysis of gene expression in pre-treatment RA samples stimulated with LPS and R848 (an imidazoquinoline agonist of Toll-like receptors (TLRs) 7 and 8) when compared to healthy controls showed significant changes in TNF family and mTOR signaling. Moreover, treatment with methotrexate for three months significantly shifted gene expression under non-stimulated and LPS and R848 stimulated conditions.

F430. Erythroid differentiation regulator 1 (Erdr1) improves wound healing of acne skin, an inflammatory skin disease.

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Acne is a chronic skin disease caused by a symbiotic microorganism, *Propionibacterium acnes* (*P. acnes*). There are numerous treatments for acne, but the development of effective therapies is still necessary. Erythroid differentiation regulator 1 (Erdr1) has been proposed to be advantageous in inflammatory skin disorders such as rosacea and psoriasis. In this study, we first showed that Erdr1 was lowly expressed at acne skin compared to normal skin, and it indicated that Erdr1 expression was negatively regulated in acne skin. To further evaluate the effects of Erdr1, it was subcutaneously injected into a mouse model of acne. The results represented that Erdr1 significantly reduced the necrotic lesions in acne skin and also decreased the infiltration of inflammatory cells. In addition, collagen synthesis and fibroblast activation were induced by Erdr1 around acne infected skin. *In vitro* experiments had established that Erdr1 accelerated collagen production in *P. acnes*-treated human dermal fibroblasts through TGF-β/Smad signaling. Moreover, Erdr1 not only down-regulated the inflammatory cytokine increased by *P. acnes* in hDF and HaCaT cells but also diminished the production of IL-8 in the monocytic cell line THP-1. In conclusion, Erdr1 promoted wound healing of

acne-infected skin via activating fibroblasts to enhance collagen synthesis and reducing inflammatory cytokine production, suggesting its potential as an acne treatment.

F437. Role of the Treg cells Induced by Excreted/Secreted Molecules of Taenia crassiceps in the Development of Experimental Colitis

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Inflammatory bowel diseases (IBD) are attributed to dysregulated immune response against intestinal bacteria in susceptible individuals. A deficiency in regulatory T cells (Tregs) has been reported to be associated with being prone to IBD. Helminths and their antigens have important immunomodulatory activities such as Taenia crassiceps and its excreted / secreted molecules (TcES) however, it is unknown if TcES generate Treg, which modulate colitis. Therefore, the aim of this study was to evaluate whether TcES alter the recruitment and activation of Treg in experimental colitis. We induced colitis with 4% DSS and daily inoculation of TcES in female C.Cg-FoxP3tm1Tch / j mice, the severity of colitis was evaluated by weight loss, DAI and histopathological damage. Flow cytometry of $CD4^+$, $CD25^+$, $FoxP3^+$, $PD1^+$, $Tim3^+$, $CD103^+$ in peritoneum, spleen, mesenteric nodules and lamina propria were evaluated. We found that TcES increases the percentage of CD4+Foxp3+ T cells in mitogen stimulated splenocyte culture. We demostrated that mice with colitis lose weight quickly and had greater histological damage with neutrophil infiltrate compared to those treated with TcES. Interestingly, the TcES increased the percentage and absolute number of Treg cells in the peritoneal cavity, also it increases the expression of CD103, PD1 and Tim3 in Treg cells, indicating that the Treg cells generated by the TcES have a greater suppressive capacity. On the other hand, the TcES modulated the CD8 population by increasing the expression of CD103. Our results demonstrated the ability of TcES to generate and modulate Treg and CD8 activation in colitis.

TH19. BCR signaling in lymphoid tissues regulated by the long isoform of Ceacam1

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Background & Aim: It has been shown that the long isoform of carcinoembryonic antigen-related cell adhesion molecule 1 (Ceacam1) expressed in T cells regulates intestinal mucosal homeostasis. We have observed higher Ceacam1 expression on B cells than T cells, but the specific Ceacam1 isoform in B cells of the gut-associated lymphoid tissues (GALT) is unknown. We analyzed expressions of each isoform in B cells in GALT and determine whether such Ceacam1 expression regulates immune responses.

Methods & Results: Semi-quantitative PCR of B cells isolated from spleen, mesenteric lymph nodes and Peyer's patches of C57BL6 revealed that the expression of the long isoform of Ceacam1 containing ITIM was dominant rather than the short form, which lacks ITIM. Confocal microscopy revealed Ceacam1 aggregation and co-localization with the B cell receptor (BCR) when B cells were activated with anti-μ antibody (Ab). When isolated B cells were stimulated with anti-μ in the presence or absence of agonistic anti-Ceacam1 Ab, BCR signal-induced cytokine productions, such as IL-4 and IL-5, were specifically suppressed by Ceacam1 signaling. Inducible overexpression of Ceacam1 in a murine B cell line, A20, showed decreased expressions of activation surface markers such as CD69 and CD80/86. This was associated with less Ca^{2+} influx and suppressed cytokine production by the overexpression of Ceacam1 after BCR signal activation.

Conclusion: These results imply that Ceacam1 regulates both B cells and T cells in GALT, and it can potentially be a therapeutic target in the management of inflammatory bowel diseases.

TH25. Increased Circulating Oncostatin M Serum Levels in Crohn's Disease Subjects Have Minimal Correlation to Disease Severity (CDAI or SES-CD) as Measured by a Newly Developed Electrochemiluminescent Assay

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Oncostatin M (OSM) is a secreted, pleiotropic, interleukin-6 family cytokine member which plays key roles in biological homeostasis and chronic ailments such as IBD. Recently, tissue gene expression levels of OSM have been predictive of disease severity and treatment outcome for TNF-neutralizing therapy in Crohn Disease (CD). Such observations suggest its potential use as a biomarker or therapeutic target. Less invasive methods than gene expression evaluation in tissue biopsies are warranted, thus we evaluated circulating levels of OSM in CD serum and its association to disease severity.

In our hands, commercial ELISAs failed traditional characterization in several human matrices, prompting in-house development of an OSM electrochemiluminescent assay. Best methodology practices required the identification of suitable antibody partners, diluents, and blocking conditions. Following completion, we assessed spike recoveries, dilution linearity, and analyte specificity. Sensitivity of the assay was in the low pg/ml range (LLOQ 16pg/ml and LOD 1.7pg/ml). We applied this new assay to a set of healthy volunteer and CD sample sets with corresponding disease activity indices. We demonstrated that OSM levels in CD subjects were significantly elevated compared to healthy volunteers (p = 4.4e-09, Mann-Whitney Test), supporting the hypothesis that OSM is dysregulated in autoimmune disorders of the gastrointestinal tract. However, OSM levels had only weak correlation with disease severity, indicated by CDAI or SES-CD scores (Spearman's rho = 0.11 and 0.28).

In conclusion, serum OSM levels, measured by this new robust assay, showed a statistically significant increase in CD subjects over healthy, with minimal correlation to disease severity.

TH29. Validation of a CRISPR/cas9-based technology platform for examining specific immune gene functions in an experimental murine model of IBD

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Inflammatory bowel diseases (IBD) are complex, multifactorial disorders characterized by chronic relapsing intestinal inflammation. Association studies have identified hundreds of genes that are linked to IBD and potentially regulate its pathology. The further dissection of the genetic network underlining IBD pathogenesis and pathophysiology is hindered by the limited capacity to investigate the role of each GWAS association through functional studies, including the generation of knockout animal models for each of the associated genes. The CRISPR/Cas9 system represents a cutting edge technology which has the potential to transform the field of IBD research by facilitating the introduction of genetic alterations in an efficient and effective manner. Using the CD40-mediated-colitis model, our results demonstrate the validity of a CRISPR/Cas9-based platform as a tool for the validation of target genes or interference strategies in experimental IBD. The utilization of this discovery strategy will allow for the timely *in vivo* validation of therapeutic targets as the rapidly emerge from current genetic and genomics efforts with human disease tissue. As such, the CRISPR/Cas9-based platform can significantly shorten the time span between target identification and generation of proof of principle experiments for drug discovery.

TH56. Pharmacological Inhibition of PARP Ameliorates Acute Lung Injury Associated Cognitive Function

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Acute lung injury (ALI) survivors have been reported to present chronic cognitive deterioration. We have recently shown that 'two hit' (HCl + LPS) induced ALI in mice results in cognitive dysfunction through the induction of systemic inflammation. The present study was designed to explore the potential anti-inflammatory effects of Olaparib, a pharmacological inhibitor of poly (ADP-ribose) polymerase on ALI mediated cognitive impairment. Olaparib was administered at dose of 5mg/kg body weight (i.p.) 30 min before each hit. Data show that olaparib pre-treatment resulted in marked reduction in the neutrophil infiltration, alveolar capillary damage and production of inflammatory cytokines (TNF-α/IL-1β/IL-6) in lungs at 24 h post ALI. Further, reduction in lung inflammation by the drug was associated with amelioration of ALI associated cognitive impairment as assessed by Morris water maze test on weekly basis. Further, restoration of cognitive function was associated with normalization of serum levels of TNF-α/IL-1β and improved the BBB function, as reflected by data on expression of occludin/claudin-5 and extravasation of Evans-blue/FITC dextran in hippocampus at 1 week post injury. Next, apparent NF-kB activation along with enhanced expression of VCAM-1, TNF-α and IL-1β in hippocampus suggests existence of neuro-inflammation, which was found to be resolved upon olaparib administration. Further, olaparib treatment 1 week after ALI induction blunted the systemic inflammation which was associated with improved BBB and cognitive function. Altogether, our results showed that olaparib protects against ALI and associated cognitive deficits in mice, and thus may offer a new treatment avenue in the area.

TH70. Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) Is Characterized By a Novel Subset Of Monocytes With Markers Associated With Crossing The Blood Brain Barrier (BBB)

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Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) is characterized by abrupt onset of obsessive-compulsive disorder (OCD) and/or food restriction with other specified neuropsychiatric symptoms. 65% of PANS patients undergo repeated episodes of flare and remission and 20% develop a chronic-static illness. In 2015, Frankovich reported monocytosis during PANS. We have followed up this observation by characterizing monocytes in active and inactive PANS. Human monocytes in blood can be divided into 3 subsets, the largest (~85%) being $CD14^+CD16^-$ "classical" monocytes. We found that $CD14^+$ monocytes were increased in all PANS flare samples and reduced during remission. We found a lower frequency of $CD14^+CCR2^+CX3CR1^+VLA\text{-}4^+CD166^+$ cells during PANS flare versus remission. CSF from new acute-onset PANS contained $CD14^+$ monocytes with these markers, suggesting an increased extravasation of these monocytes across the BBB. These monocytes were increased in frequency in blood samples from PANS remission, suggesting they may have an immunosuppressive function. In chronic-static PANS patients, the frequency of these monocytes in the circulation was low, and they were undetectable in CSF, suggesting low production during chronic PANS patients. Incubation of healthy PBMC with plasma from PANS flare led to an increase in the expression of CCR2, CX3CR1, VLA-4, Iba1, and HLA-DR, suggesting soluble mediators drive this phenotype. Additionally, we found an increase in circulating, inflammatory monocytic DC ($CD14^+CD11c^+CD209^+$) in PANS flare, suggesting this myeloid cell type contributes to inflammation. Together, our data support a model in which myeloid cell subsets contribute to persistence and suppression of PANS brain inflammation.

TH99. Adalimumab Induces Wound Healing Profile in Patients with Hidradenitis Suppurativa by Regulating Macrophage Differentiation and Matrix Metalloproteinase Expression

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BACKGROUND: Adalimumab (ADA) is the first FDA-approved treatment for moderate-to-severe hidradenitis suppurativa (HS). Etanercept (ETN) has been shown to be ineffective for treating HS, suggesting that the mechanism of action (MOA) of ADA is distinct in HS.

METHODS: A macrophage differentiation assay was carried out to assess the role of TNF-anti-TNF complexes in macrophage differentiation. To investigate if a systemic wound healing phenotype correlates with adalimumab response, circulating matrix metalloproteases (MMP) expression was examined for HS patients in PIONEER clinical trials.

RESULTS: TNF-ADA complexes were significantly more effective in preventing the TNF-induced effects than TNF-ETN complexes *in vitro*. TNF-ADA complexes also exhibited stronger inhibitory effects on inflammatory macrophage differentiation by diverting these cells towards a wound healing phenotype. Moreover, RNA sequencing and Ingenuity pathway analysis revealed unique regulatory profiles for TNF-ADA-treated inflammatory macrophages, which were not observed for those treated with either TNF-ETN or TNF-CZP, including inhibition of MMP pathway. In addition, adalimumab inhibits inflammatory MMP expression while promoting would healing MMPs in the circulation of HS patients who respond to ADA treatment.

CONCLUSIONS: Our *in-vitro* findings demonstrate that a distinct wound healing profile may be present in TNF-ADA-treated inflammatory macrophages, suggesting a possible MOA of ADA in HS wound healing. Moreover, ADA not only differentially regulates MMP expression in HS patients responding to the therapy but potentially induces a transition to a wound healing profile. Additional evaluation of wound healing parameters will be performed to validate the effects of adalimumab in the SHARPS clinical trial.

TH115. Identification and Characterization of Flagellin-reactive CD4 T cells among Crohn's Disease Patients

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CD4 T cells display aberrant reactivity to certain flagellin antigens (CBir1, Fla-2, and Fla-X) in Crohn's patients not similarly seen in healthy controls. Yet, little is known about the frequency, functionality, and phenotype of flagellin-reactive T cells in Crohn's patients. This study aims to identify flagellin-reactive $CD4^+$ T cells in Crohn's patients' peripheral blood and to test the hypothesis that there is decrease CD4 T cell responses to flagellin antigens during Crohn's disease remission. To detect antigen-reactive cells we magnetically enriched surface CD154 on $CD4^+$ T cells and assessed intracellular CD154 and cytokine production by flow cytometry. In Crohn's patients (N=40) we found 0.001-0.008% of the total $CD4^+$ T cells post-enrichment for CD154 expression are specifically reactive to flagellin antigens. These CBir1-, Fla-2-, and Fla-X-specific-$CD154^+CD4^+$ T cells produced significantly higher TNF-α, IFN-γ and IL-17A as compared to healthy controls. However, assessment of CD4 T cell responses to flagellin antigens among active (N=20) and remission (N=20) Crohn's patients showed no significant difference in CD154 expression profiles and cytokine production. We next examine whether flagellin-reactive $CD4^+$ T cells from remission and active patients similarly disrupt epithelial barrier function. We cultured HT-29 cells with supernatants from flagellin-treated cell cultures and measured Claudin-2 expression by qPCR. Interestingly, supernatants from flagellin-treated cultures induced higher Claudin-2 expression using cells from active versus remission Crohn's patients. Overall our data demonstrate that flagellin-

activated CD4 T cells in Crohn's remission fail to induce epithelial barrier changes despite similar activation profiles and frequency to CD4 T cells from active patients.

TH153. Retrospective Polymyositis Case Series

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A Retrospective Polymyositis Case Series

The inflammatory myopathies represent a rare group of muscle disorders. Herein we report our experience in 14 patients diagnosed with polymyositis over the past 4 years. Autoantibody determinations revealed 7 with positive antinuclear antibody (ANA), 4 with Jo-1 antibody (3 also ANA+), 1 with signal recognition particle antibody (SRP+), 1 positive for 3-hydroxy-3-methylglutaryl-coenzyme A reductase antibody and 5 were autoantibody negative. Severe interstitial lung disease (ILD) was seen in 2 ANA + patients. Three of four patients with Jo-1 positivity exhibited mild or equivocal ILD. Severity of disease was defined as 1: bedridden/wheelchair bound/swallowing impairment/respiratory compromise) #6, 2: required hospitalization but became ambulatory #4 and 3: ambulatory #3. One patient could not be characterized. 11 patients required hospital admission. One required intubation.Treatment: Steroid therapy in excess of the recommended 3 day high dose bolus tapered to 1 mg/kg/day (methylprednisolone or prednisone) was required for 4 patients with disease severity 1. Twelve patients were co-treated with mycophenolate, 2 were treated with azathioprine. 8 patients were treated with rituximab and 3 received at least one course of intravenous immunoglobulin. Two patients with severe ILD came under control easily with 3 monthly cyclophosphamide infusions (1 gram/m2).**Outcome**: Three patients were lost to follow-up. The SRP+ patient continues with active debilitating disease. Ten patients were in complete remission or low disease activity. One patient suffered a pulmonary infection during hospitalization.**Conclusion**: Our experience suggests aggressive use of steroid sparing agents limits adverse steroid side effects and offers good prognosis.

TH172. Anti-GM-CSF Autoantibodies: Predictors of Crohn's Disease Severity and a Novel Therapeutic Approach

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Crohn's disease (CD) is a severe inflammatory disorder of the gastrointestinal tract, and is currently not curable. CD is multifactorial, suspected to result from a combination of genetic and environmental risk factors leading to dysregulated intestinal immune homeostasis. Mononuclear phagocytes (MNP) are key innate immune cells that support intestinal homeostasis through facilitating immune tolerance, antimicrobial activity, and barrier integrity. A crucial cytokine that promotes the survival and function of intestinal MNP is Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF). Interestingly, anti-GM-CSF autoantibodies can be detected in the serum of almost 1 in every 5 CD patients and correlates with severe and complicated disease manifestation. Our data demonstrates that anti-GM-CSF autoantibodies precede the onset of CD up to 3000 days. Further characterization revealed a unique isotype and epitope profile of these antibodies compared to anti-GM-CSF autoantibodies described in patients suffering from pulmonary alveolar proteinosis (PAP). Moreover, we demonstrated that these anti-GM-CSF autoantibodies neutralize the bioactivity of GM-CSF on MNP by binding to post-translational modifications, suggesting a role for these autoantibodies in promoting intestinal immune dysregulation. Here, we provide a strategy to generate recombinant human GM-CSF variants that selectively escape recognition by CD-specific anti-GM-CSF autoantibodies. Our results could enable a predictive assay and a novel therapeutic approach to prevent the onset of severe and complicated disease manifestation in a subgroup of CD patients.

TH369. Beneficial effects of Gallic acid in amelioration of COPD associated Exacerbation in mice

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Chronic Obstructive Pulmonary Disorder (COPD) is an inflammatory lung disease, which is often exacerbated with microbial infections resulting in worsening of respiratory symptoms. Gallic acid (GA), a naturally occurring phenolic compound is known to possess anti-oxidant and anti-inflammatory activities. Additionally, we have recently reported that GA protects against the elastase (ET) and cigarette smoke (CS) induced inflammation and emphysema and the present work was designed to investigate the beneficial effects of GA against ET+LPS induced COPD exacerbation like condition in mice model. Our data showed that LPS administration at 21 days after ET instillation resulted in significant infiltration of inflammatory cells particularly neutrophils and elevated levels of pro-inflammatory cytokines (TNF-α/IL-1β/IL-6) in the lungs. Interestingly, daily administration of GA (200 mg/Kg b. wt.) starting 7 days before ET instillation, blunted the ET+LPS induced inflammation as indicated by reduced number of BALF inflammatory cells particularly neutrophils along with suppression of myeloperoxidase activity and production of pro-inflammatory cytokines. Further, GA also restored the redox imbalance in the lungs towards normal. Protection conferred by GA was accompanied by marked increase in protein levels of Nrf2 with concomitant increase in transcription of its downstream targets HO-1/Prdx-1. Conversely, phosphorylation of p65-NF-kB was found to be reduced, which was associated with down-regulation in the gene expression of IL-1β/TNF-α. Overall, our data show that GA effectively modulates COPD exacerbation manifestations in mice.

TH372. RPT193, A Small Molecule CCR4 Antagonist, Effectively Inhibits Chemokine Ligand-Binding On Th2 Cells In A First-In-Human Healthy Volunteer Study

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Atopic dermatitis (AD) is an inflammatory skin disease characterized by dysregulation of the T helper type 2 (Th2) cell response. The chemokine receptor CCR4 is highly expressed on Th2 cells and initiates CCL17- and CCL22-mediated chemotaxis to inflamed tissues. We developed an oral, small molecule CCR4 antagonist, RPT193, to treat AD and other allergic inflammatory diseases. To quantify CCR4 target engagement on human Th2 cells, we developed a CCR4 receptor occupancy assay for *ex vivo* analysis of human whole blood. We demonstrate that the assay meets intra-assay and inter-assay reproducibility. Target engagement of CCR4 on Th2 cells was assessed in healthy volunteers as part of an ongoing, double-blind, placebo-controlled healthy volunteer Phase 1 study. Four hours following a single 100mg dose of RPT193, all subjects achieved a loss of CCL22 binding that is concordant with the pre-clinically determined CCR4 inhibition necessary to prevent human Th2 chemotaxis *in vitro*. Twenty-four hours after the 100mg dose of RPT193, 5 of 6 subjects maintained the targeted loss of CCL22 binding. In summary, we have demonstrated that all healthy volunteers receiving a single 100mg dose of RPT193 achieved the targeted decrease in CCL22 binding. We hypothesize that this level of CCR4 inhibition will effectively reduce Th2 migration into inflamed tissues and ameliorate Th2-driven allergic diseases such as AD. RPT193 is currently being evaluated in a dose-expansion cohort of this ongoing Phase 1 study for safety, tolerability and early clinical activity in patients with moderate to severe atopic dermatitis (Clinicaltrials.gov ID: NCT04271514).

TH373. Plasma-derived extracellular vesicles as novel biomarkers of disease activity and clues to pathogenesis in systemic juvenile idiopathic arthritis

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Background: Systemic juvenile idiopathic arthritis (sJIA) is a chronic pediatric inflammatory disease of unknown cause. The biologic mechanisms driving sJIA are incompletely characterized. We hypothesize that the number and cellular sources of extracellular vesicles (EVs) differ between inactive and active states of sJIA and relative to healthy controls.

Methods: We evaluated plasma from healthy pediatric controls (n=13) and sJIA patients with active systemic flare (n=15) or inactive disease (n=12). We determined total EV abundance and size

distribution using microfluidic resistive pulse sensing following EV isolation by size-exclusion chromatography. Cell-specific EV subpopulations were measured by high-resolution flow cytometry.

Results: Total EV concentration did not significantly differ between controls and sJIA patients. EVs with diameters < 200 nm were the most abundant and the majority of cell-specific EV subpopulations were within this smaller size range. Relative to controls, sJIA patients had significantly higher levels of EVs from platelets, monocytes, myeloid and endothelial cells. Differences were most pronounced in doubly stained EV populations from activated platelets, intermediate monocytes, and chronically activated endothelial cells, latter two of which were significantly more elevated in sJIA flare relative to inactive disease and controls.

Conclusions: The observation of elevated EVs from specific cellular sources and activation states suggest that EVs play a role in modulating immune activity in sJIA. Our findings indicate that multiple cell types contribute to altered circulating EV profiles in sJIA. The EV differences between sJIA disease states and healthy controls implicate EV-mediated cellular crosstalk as possibly driving sJIA activity.

TH376. An IL2RA enhancer polymorphism determines responsiveness to IL2 signalling and the balance between pathogenic and protective T cell immunity in Crohn's disease

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Introduction:

Differential responsiveness to interleukin (IL)-2 between effector $CD4^+$ T cells (T_{eff}) and regulatory T cells (T_{reg}) is a fundamental mechanism of immunoregulation, with T_{reg} an order of magnitude more sensitive to IL-2 signalling. The single nucleotide polymorphism (SNP) rs61839660 lies within an *IL2RA* intronic enhancer bound by the transcription factor T-bet, and is causal in Crohn's disease (CD).

Method:

Blood samples from CD patients and healthy controls homozygous minor allele (TT), homozygous major allele (CC), or heterozygous (CT) were obtained from the National Institute of Health Research Inflammatory Bowel Disease Bioresource. T_{reg} and T_{eff} were sorted by flow cytometry and mean fluorescence intensity of IL2-R (CD25) analysed. We assessed the extent of T_{eff} and T_{reg} STAT5 phosphorylation (pSTAT5) in the three groups following IL-2 exposure.

Results:

There were no significant differences in disease phenotype/behaviour between patient groups. TT T_{eff} demonstrated increased CD25 expression, with a reduced ratio of T_{reg}/T_{eff} CD25 expression in these subjects. Both CT and TT subjects exhibited enhanced T_{eff} pSTAT5 activation on stimulation with IL-2.

There was no difference in CD25 expression or pSTAT5 response between patients and controls of each genotype.

Conclusion:

Minor allele homozygosity of rs61839660 confers a functional 'hyper-responsiveness' to IL-2 signalling due to increased T_{eff} CD25 expression. Reduced fold-difference in CD25 expression between T_{reg} and T_{eff} impedes the ability of T_{regs} to preferentially respond to low doses of IL-2 in these subjects, meaning activated T_{eff} drive inflammation. This work paves the way for therapeutic trials of CD25 blockade in genetically stratified individuals.

TH385. Estimation of IL-18 levels in newly diagnosed type 2 diabetes mellitus

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Type 2 diabetes mellitus (T2DM) is a multifactorial disorder in which genetic, lifestyle changes and environmental factors are involved, in the development of insulin resistance, leading to hyperglycemia. Previous studies have reported the role of inflammation in the pathogenesis of type 2 diabetes mellitus. This has increased the significance of inflammatory markers as prognostic biomarkers of early diagnosis and progression of the disease. Several cytokines, such as IL-2, IL-6, and IL-18 are observed to be associated with type 2 diabetes mellitus. Some studies have reported a plausible association of IL-18 with type 2 diabetes, but the data is unclear and inconsistent. The present study aimed to compare IL-18 levels in newly diagnosed type 2 diabetes mellitus patients with non-diabetic controls. 35 newly diagnosed diabetic cases and 35 non-diabetic controls were recruited after obtaining due informed consent. Venous whole blood was collected under aseptic conditions. Biochemical parameters were analyzed using the autoanalyzer. Serum levels of IL-18 were performed using a commercially available ELISA kit. The fasting blood sugar levels and glycated hemoglobin were significantly higher in T2DM patients than controls ($p< 0.001$). The mean ± SD of IL-18 was significantly higher ($p=0.0068$) among cases when compared to controls (696.9 ± 282.1 pg/ml and 524.4 ± 171.9 pg/ml respectively). Findings from the present study suggest the pro-inflammatory role of IL-18 in newly diagnosed Type 2 Diabetes Mellitus.

TH444. Anti-angiogenic effect of an antimicrobial and anti-inflammatory peptide isolated from the skin of the Mexican tree frog, Pachymedusa dacnicolor

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Psoriasis is considered a chronic inflammatory skin disease, which affects between 0.09% and 11.4% of the global population, and constitutes a problem for public health. In psoriasis and other similar inflammatory skin diseases, biological processes such as inflammation and infection play a major role in skin lesion's progress. Particularly, pathogenic angiogenesis is a significant part of the inflammatory process, with fibroblasts and inflammatory cells as critical components of the development of this pathogenic angiogenesis.

We isolated a peptide from the Mexican tree frog *Pachymedusa dacnicolor* that presents antimicrobial properties and modulates the pool of inflammatory cells at micromolar concentrations.

Here we demonstrate, in the *ex vivo* aortic ring model and *in vitro*, that this peptide inhibited neo-vessels formation by inducing the death of endothelial cells and fibroblasts. However, the peptide did not affect the vessels already established. Moreover, in the *in vivo* mouse model of psoriasis induced by Imiquimod, the peptide inhibited the formation of neo-vessels locally. Interestingly, our peptide was much more efficient in inhibiting angiogenesis than clobetasol propionate, a corticosteroid used in psoriasis treatment.

Innate immunity

F113. Autophagy-related protein VPS34 controls the homeostasis and function of macrophages

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Vacuolar protein sorting 34 (VPS34; also known as PIK3C3) plays a role in both canonical and noncanonical autophagy, key processes that control immune cell responsiveness to a variety of stimuli. Our previous studies found that VPS34 is a critical regulator that controls the development, homeostasis, and function of dendritic cells and T cells. In this study, we investigated the role of VPS34 in macrophage biology using myeloid cell-specific *Vps34*-deficient mice. We found that *Vps34*-deficient macrophages express increased surface levels of MHC class I and class II molecules. Interestingly, *Vps34* ablation in macrophages caused a partial impairment in the homeostatic maintenance of TIM-4$^+$ macrophages and defective uptake of apoptotic cells. In addition, myeloid cell-specific *Vps34*-deficient animals showed significantly reduced severity of experimental autoimmune encephalomyelitis (EAE), a primarily CD4$^+$ T cell-mediated mouse model of multiple sclerosis. Importantly, peritoneal macrophages

from mice deficient in the VPS34-associated protein RUBICON, which is critical for a noncanonical form of autophagy called light chain (LC) 3-associated phagocytosis (LAP), showed normal MHC class I, MHC class II, and TIM-4 expression and *Rubicon*$^{-/-}$ mice developed signs of EAE similar to wild-type control mice. These results suggested that the canonical autophagy-dependent activities of VPS34 play a critical role in controlling macrophage homeostasis and function. Collectively, our studies establish VPS34 as an important regulator of macrophage functions and macrophage-mediated regulation of EAE. Our findings also have important implications for the development of small-molecule inhibitors of VPS34 for therapeutic purposes.

F199. Integrated Cross-species Analysis Identifies a Conserved Transitional Dendritic Cell Population

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Plasmacytoid dendritic cells (pDCs) are sensor cells with diverse immune functions, from type-I interferon (IFN-I) production to antigen presentation, T cell activation, and tolerance. . Regulation of these functions remains poorly understood but could be mediated by functionally specialized pDC subpopulations. Recently, we and others described a novel population of human DCs contained within previous pDC descriptions. We called these cells transitional DCs (tDCs) given their heterogeneous expression of pDC and classical DC (cDC) features. To identify the mouse homolog of this population, we now performed an integrated cross-species analysis using CyTOF, transcriptomics and functional assays. Our analysis shows that, similar to humans, mice harbor a population of DCs with a pDC-like to cDC-like phenotype, i.e., mouse tDCs. Mouse and human tDCs express transcription factors essential for both pDC (Tcf4) and cDC (Irf8, Irf4) development. However, similar to pDCs, tDC development is dependent on Tcf4 and not Irf8. Additionally, tDCs express features that are characteristic of pDCs and absent from cDCs. *In vitro* assays demonstrated that tDCs are specialized in antigen presentation and have limited capacity for IFN-I production. Finally, in a model of murine respiratory viral infection, tDCs and pDCs showed similar recruitment dynamics to the lung. Altogether, we conclude that tDCs are an evolutionarily conserved DC population that is closely related to pDCs. Our identification of mouse tDCs provides a framework for deciphering the function of pDCs and tDCs during diseases, which has the potential to open new avenues for therapeutic design.

F270. Impact of Exposure to Ambient Particulate Matter 2.5 on Frequency and Function of Circulating Monocyte Subsets in Children

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The impact of exposure to air pollution on immune system is unclear. In particular, association between particulate matter with a diameter of less than 2.5 micrometers (PM2.5) and circulating monocytes in children has not been reported. We aim to investigate whether living in an area with increased PM2.5 is

associated with alteration of circulating monocytes. We further study whether pollution-dysregulated monocytes are associated with clinical measures of asthma.

We recruited healthy and asthmatic children from Fresno, California, an area with elevated air pollution. Using cytometry time-of-flight (CyTOF), we characterized phenotypic and functional markers specific to monocyte subsets in PBMC. CyTOF data were analyzed by R programming and further validated by manual gating. Furthermore, we measured markers of inflammation in the plasma using Luminex 63-plex assay.

Increased exposure to PM2.5 was associated with elevation of classical monocytes (CM) in children (p=0.03). CM from high PM2.5 exposed children upregulate nuclear expression of aryl hydrocarbon receptor, AhR (p=0.05). Decline in FEV1/FVC was associated with increased accumulation of CM in children. A significantly negative correlation between FEV1/FVC and frequency of CM was observed (p=0.01, r=-0.72). Individual marker clustering demonstrated that increase in plasma level of IL-1b is associated with elevation of AhR in CM (p=0.01).

Increased frequency of CM as well as upregulation of IL-1b and AhR upon air pollution exposure suggest a potential inflammasome-mediated mechanism by which monocyte functions could be dysregulated. Increased accumulation of CM in asthmatic children may represent a novel immune signature as a prognostic biomarker in highly polluted areas.

F393. Endothelial Costimulatory Phenotype is Durably Altered following IFNγ or TNFα exposure

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We characterized the repertoire of cytokines, antigen presentation and costimulatory molecules produced by inflamed vascular endothelial cells (EC), under chronic stimulation and after cytokine withdrawal.

EC were stimulation with TNFα or IFNγ for 1-24hr. mRNA were measured by Nanostring; cell surface and secreted protein were measured by flow cytometry, ELISA and Luminex.

TNFα triggered IL6, IL6ST, IL15, TRAIL and BAFF production from EC as a late phase (>6hr) response, while IFNγ stimulated only TRAIL, BAFF and IL15. Constitutively expressed CXCL12 and IL32 were downregulated. EC upregulated CD40, ICOSL, HVEM, PD-L1, PD-L2 and 4-1BB, and down-regulated B7-H3, in response to TNFα; while IFNγ increased HVEM, PD-L1 and PD-L2. TNFα augmented expression of HLA I, TAP1 and proteasome components; while IFNγ also increased HLA II, CIITA, and CD74.

After short (3hr) cytokine priming and extended withdrawal from cytokine exposure, TNFα-induced HLA I, IL6ST and PD-L2 were enhanced at 24hr. Other TNFα-induced costimulatory molecules and cytokines rapidly contracted. In contrast, HLA I and proteasome induction at 18hr and 24hr was equal whether IFNγ was chronic or withdrawn. HLA II expression was also elevated, albeit lower than with continuous IFNγ. In addition, nearly all IFNγ-induced costimulatory molecules and cytokines persisted at intermediate levels after IFNγ withdrawal.

In summary, our data suggest that chronic stimulation of EC with TNFα is needed to promote a late phase costimulatory phenotype, while only 3hr exposure to IFNγ provoked a prolonged endothelial phenotype change, with markedly sustained HLA I more than one day after IFNγ was last present.

F446. Features of Mast Cell Activation in patients with mannose binding lectin deficiency

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Mast cell activation (MCA) disorders have been described, including rhinitis, asthma, urticaria and anaphylaxis. Mast cell activation syndrome (MCAS) is defined by the combination of 1) typical symptoms, 2) response to treatment, and 3) and laboratory values.

Mannose-binding lectin (MBL) is a component of the innate system, with 7% of the population deemed MBL deficient. An association with between MBL deficiency and MCA disorders has never been studied.

Methods: This is a retrospective study of patients, seen in a single Immunology practice, using published criteria for MCAS diagnosis: typical symptoms, response to anti-MC agents, and validated MCA markers. Medical histories were analyzed for MCA and PID associated illnesses. Immunological tests included measurements of MBL, complement, immunoglobulin, and immune cell populations. Validated markers of MCA, included the following: serum tryptase and histamine; urine N-methylhistamine and prostaglandin metabolites.

Results: 25 patients with MBL deficiency was identified, with most presenting with classic MCA symptoms, including asthma, urticaria, and hypersensitivity gastroenteritis. 2/3 MBL deficient patients reported relief of these symptoms to MC targeted medications, including anti-histamines. 1/3 of patients had a positive test of validated MCA marker. 14/25 had past medical histories worrisome for possible PID, including recurrent sinopulmonary infections. Pneumococcal immunity was depressed in 60% of patients.

Conclusions: Recent studies suggest that PID can present with hypersensitivity syndromes, including autoimmune cytopenias and endocrinopathies. This study highlights features of MCA in MBL deficient patients, indicating the need to expand the list of warning signs of PID, to include symptoms of MCA.

TH15. Long-Term Outcomes and Treatment Efficacy in Patients with TNF Receptor-Associated Autoinflammatory Syndrome (TRAPS) from the Eurofever International Registry

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Objectives. To define best treatment approach in patients with Tumour necrosis factor Receptor-Associated Periodic Syndrome (TRAPS) and effect on long-term outcomes. **Methods**. We reviewed all data on patients with TRAPS enrolled in the Eurofever international registry according the new INSAID gene variant classification and the new Eurofever/PRINTO classification criteria (EPCC). **Results**. Data on 226 patients were available. Patients not fulfilling the EPCC carrying likely benign/benign variants (21 patients, 9%) or VOUS/not classified variants (40 patients, 18%) displayed a milder disease than the patients fulfilling the EPCC with VOUS/not classified variants (38 patients, 17%) or pathogenic/likely pathogenic variants (127 patients, 56%). In particular, less frequent abdominal pain and skin rashes, higher efficacy rate of colchicine as maintenance therapy and no development of AA amyloidosis have been reported in patients not fulfilling the EPCC. Almost 90% of patients fulfilling the EPCC required maintenance therapy. Anti-interleukin (IL)-1 drugs were the most frequently used, with the highest efficacy rate (>85% complete response), while Etanercept was less effectively used and discontinued in 65% of patients. No patients on anti-IL-1 treatment developed amyloidosis and seven women with history of failure to conceive had successful pregnancies following complete disease control with anti-IL-1 drugs. **Conclusions**. Anti-IL-1 drugs are the best maintenance treatment in TRAPS with potential to reverse the most serious disease complications of AA amyloidosis and infertility. The diagnosis of TRAPS should be considered very carefully in patients carrying VOUS/not classified variants not fulfilling the EPCC.

TH20. Complement component C5 is present on normal human kidney microvascular endothelial cells

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The traditional view of complement is as an innate immune system composed of an extracellular cascade of proteins made by the liver, which when activated function in opsonization, chemotaxis and cell lysis. More recently it has been suggested that complement proteins may be made locally by cells, particularly leukocytes, and function in physiologic processes.

We used eculizumab, a monoclonal antibody to intact C5, with flow cytometry to investigate localization of C5 within native and transplanted human kidneys. To our surprise, eculizumab bound to microvascular endothelial cells at a level 1-2 logs above a control IgG4 antibody even in normal kidneys. Binding was higher in transplanted kidneys with rejection. In transplanted kidneys with antibody mediated rejection, eculizumab and anti-lambda and –kappa were present on the same endothelial cells. Our technique also identified intact C5 on the surface of intra-renal and peripheral blood leukocytes.

Finding eculizumab binding to kidney microvascular endothelial cells under conditions without inflammation, we then assessed the binding of complement inhibitory proteins. CD59 and CD55 (decay-accelerating factor) were present at levels higher than on leukocytes.

Our results suggest human kidney microvascular endothelial cells produce their own C5 which is available for activation when C5 convertases are present, but that damage is controlled by complement inhibitory proteins on the same cells. This differs from current views of immune kidney disease in which complement activation is thought to occur by extracellular C5 attaching to endothelial cells only after C5 convertases are bound.

TH106. Interleukin-27 gene therapy inhibits lethal autoimmunity in Scurfy mice

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We previously reported that injection of IL-27-expressing recombinant adeno-associated virus (AAV-IL-27) induced systemic depletion of regulatory T cells (Treg). However, AAV-IL-27 treatment did not lead to obvious autoimmune inflammation in mice. To test if AAV-IL-27 therapy can protect mice against Treg deficiency-caused autoimmunity, we used it to treat the Foxp3 mutated *Scurfy* mice that develop

autoimmune diseases similar to immune dysregulation, polyendocrinopathy and enteropathy X-linked syndrome (IPEX) in human. We found that a single dose of AAV-IL-27 treatment given on day 8-10 post birth, when signs of *Scurfy* phenotype appeared, dramatically extended the survival of the *Scurfy* mice and ameliorated immune pathology in various tissues. In the spleen and lymph nodes of AAV-IL-27 treated mice, AAV-IL-27 gene therapy significantly prevented naïve T cell activation as reflected by downregulation of CD62L and upregulation of CD44. AAV-IL-27 therapy promoted both IFN-g and IL-10 production in T cells, and genetic deletion of IL-10 in *Scurfy* mice resulted in mice less responsive to AAV-IL-27 therapy. Our study suggests that IL-27 gene therapy inhibits lethal autoimmunity in *Scurfy* mice and IL-27 gene therapy may be a potentially new treatment for IPEX.

TH440. Identification of a New Early Neutrophil Progenitor in Human Bone Marrow That Contributes to Neutrophil Heterogeneity in Cancer

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An expansion of neutrophils often marks cancer. Neutrophils are the most abundant peripheral immune cells and thus, are continually replenished by bone marrow-derived progenitors. Neutrophils have been shown to display both pro- and anti-tumoral functions in the tumor microenvironment. However, neutrophil production and heterogeneity remains ill-defined. We used CyTOF mass cytometry to identify a homogeneous unipotent neutrophil progenitor subset we term 'early neutrophil progenitor (eNeP)' (Lin-CD66b$^+$CD117$^+$CD71$^+$) in human bone marrow. RNA-expression analyses, together with *in vivo* adoptive humanized mouse transfers, indicate that eNeP are the earliest human unipotent neutrophil progenitors. Furthermore, we identified CD71 as a marker associated with this earliest neutrophil developmental stage. Importantly, eNeP were expanded in the blood of melanoma patients and were detectable in both blood and tumors from lung cancer patients. Functionally, we find that these cells not only function as stem cells, but also function as bona fide neutrophils. They generate NETs, secrete IL1b, contain granules, and express myeloperoxidase. Thus, these cells functionally contribute to the tumor microenvironment, and likely play a key role in regulating tumor growth and progression. Emerging immunotherapies that can regulate the numbers and/or function of eNeP could change tumor dynamics and cancer outcomes.

Leukocyte Migration

F236. GPR56 inhibits chemotaxis of human Natural Killer and TEMRA lymphocytes

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Within the human immune system, the expression of GPR56, an adhesion G-protein couple receptor, is restricted to cytotoxic Natural Killer (NK) and T lymphocytes, including T effector memory re-expressing CD45RA (TEMRA) cells. In primary NK cells, GPR56 acts as an inhibitory receptor, as it diminishes their ability to eliminate target cells. Overexpression of GPR56 in immortal NK-92 cells leads to robust inhibition of cell chemotaxis. GPR56 is speculated to promote retention of cytotoxic lymphocytes in inflamed peripheral tissues. Thus, GPR56 has the potential to regulate immune responses that may lead to excessive inflammation and autoimmunity. The aim of the present study was, therefore, to examine whether GPR56 mediates cytotoxic immune cell functions and whether GPR56 expression is altered in autoimmune diseases. To tackle these questions, we first compared expression of GPR56 in healthy and autoimmune disease patient tissues using scRNA-seq datasets. Next, we tested whether stimulation of GPR56 with a monoclonal antibody modulated the migratory capacity of NK and $CD8^+$ TEMRA cells. We found that chemotaxis toward CXCL12, IL-8, and CX3CL1, was strikingly inhibited by GPR56. Using bulk RNA-seq, we found GPR56-stimulation impacted a multitude of signaling cascades that regulate cell chemotaxis. Taken together, our results confirm that GPR56 is a marker for NK and TEMRA cells in healthy and autoimmune patient tissues and establish GPR56 as a key regulator of lymphocyte chemotaxis.

Metabolism and Microbiome

F54. Alterations of the Gut Ecological and Functional Microenvironment in Different Stages of Multiple Sclerosis

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[Objective] To reveal the characteristics of the gut microbiomes in patients with different stages of multiple sclerosis (MS). [Methods] We analyzed gut microbiomes of 62 relapsing-remitting MS (RRMS), 15 secondary progressive MS (SPMS), 21 atypical MS, 20 neuromyelitis optica spectrum disorder (NMOSD) patients and 55 healthy controls (HC) by 16S rRNA gene, shotgun metagenomic sequencing, and metabolite analysis of fecal samples. [Results] UniFrac distance analysis revealed significant dysbiosis in RRMS, SPMS, and NMOSD based on 16S data (adjusted p = 0.0099, 0.029, 0,0099, respectively). The analysis found a total of 30 species having significant changes in abundance and significant correlation with clinical severity in the four distinctive patient groups. Metagenomic gene and metabolite analysis revealed marked reduction in butyrate biosynthesis and level in RRMS compared to HC (p = 0.0007), concurrently with a significant decrease in *Eubacterium rectale* in RRMS compared to HC (adjusted p = 0.0053). Notably, our data also revealed an enhanced capacity for DNA mismatch repair in SPMS than in RRMS and a reduced capacity for carbohydrate metabolism in SPMS than in HC (adjusted p = 0.0009), suggesting excessive oxidative stress in the gut with SPMS, which was confirmed by increased ratio of cysteine and glutathione persulfide to their non-persulfide forms in

SPMS compared to HC in sulfur metabolite analysis (p = 0.0152, 0.0432, respectively). [Conclusions] The present study revealed ecological and biological alterations of the gut microenvironment in different stages of MS.

F76. Reticular Dysgenesis-Associated Adenylate Kinase 2 Maintains Energy Homeostasis and Redox Balance Indispensable for Hematopoietic Stem and Progenitor Cell Development

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Cellular metabolism plays an intricate role in directing cell fate decisions during hematopoiesis. Biallelic mutations in the mitochondrial enzyme adenylate kinase 2 (AK2), cause Reticular Dysgenesis (RD), one of the most profound forms of severe combined immunodeficiency (SCID). RD patients also suffer from severe congenital neutropenia. AK2 catalyzes the interconversion between adenine nucleotides and controls the availability of ADP for oxidative phosphorylation. The developmental arrest across multiple lineages suggests that AK2 deficiency causes a metabolic defect with global impact on cellular functions. Using a cell-traceable CRISPR model of AK2 biallelic knock-out in human HSPCs, we recapitulated the RD granulocytic maturation arrest *in vitro*. A metabolomics analysis showed that $AK2^{-/-}$ HSPCs exhibit decreased TCA cycle activities and ATP level, and an increase in AMP. In addition, ribosome biogenesis and protein translation, the most energy demanding aspects of cellular proteostasis, are compromised. Intriguingly, $AK2^{-/-}$ HSPCs also exhibit a decreased NAD^+/NADH ratio, possibly caused by reduced electron flux through the electron transport chain, where NADH is oxidized to NAD^+. This NAD^+ deficiency could explain our observation that β-oxidation is severely defective in $AK2^{-/-}$ cells, which further limits the substrate for TCA cycle and curtails ATP production. In conclusion, our data showed that the defective mitochondrial respiration in $AK2^{-/-}$ HSPCs leads to a metabolic failure in β-oxidation through sequestration of the NAD^+ pool, which ultimately causes a cellular ATP crisis with ensuing demise in ribosome and protein synthesis. Defining the molecular mechanism between energy metabolism and protein homeostasis is subject of further investigation.

F137. Correction of Underlying Lymphoid Glucose Utilization Defects with the BCG Microorganism: Implications for Type 1 and Type 2 Diabetes through Quantitative Method Development

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Human clinical trial data with 8-years of follow up shows long-lasting blood sugar control in type 1 diabetes (T1D) after BCG vaccinations. BCG-vaccinated subjects experienced lymphoid restoration of glucose metabolism through a shift from oxidative phosphorylation to aerobic glycolysis, correcting the defective glucose metabolism. We sought to determine whether individuals with type 2 diabetes (T2D) have similar underlying lymphoid defects in sugar utilization. We investigated i. baseline defects in aerobic glycolysis in T1D and T2D versus non-diabetic controls (NDC), and ii. the impact of BCG on glucose utilization *in vitro* and *in vivo*. At baseline, T1D lymphocytes showed defects in 2-NBDG uptake (indicating underactive aerobic glycolysis) and high baseline oxygen consumption (indicating overactive oxidative phosphorylation). After 24-hour exposure to BCG, both T1D and NDC lymphocytes increased sugar utilization; T1D restoration was close to normal. This was also observed *in vivo* with 3-year clinical trial monitoring of BCG-treated T1D subjects. BCG treatment of cultured T1D lymphocytes decreased oxygen consumption and increased the extracellular acidification rate (indicating increased aerobic glycolysis) in OCR and ECAR assays. In T2D lymphocytes, baseline blood sugar utilization was higher than in T1D and NDC. BCG treatment of T2D lymphocytes accelerated sugar transport to levels above NDC levels. We conclude that T1D and T2D have different levels of basal lymphoid sugar utilization compared to NDC: T1D lymphocytes have lower levels and T2D lymphocytes have higher levels. Additionally, BCG-treated T2D lymphocytes were responsive to BCG. BCG *in vitro* or *in vivo* appears to increase glucose uptake in diabetes.

TH197. GDF15 Is Not Required For Normal Osteoclast Function in Steady-State Nor in Context Of Osteoporosis

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Growth differentiation factor 15 (GDF15) is an emerging regulator of energy homeostasis. Upon binding its receptor GFRAL in the hindbrain, it gets involved in body weight regulation. GDF15 also mediates tissue tolerance against inflammation via the sympathetic output on the liver. Bone marrow derived cells can make GDF15 but how it influences their function is unclear. For example, while GDF15 was shown to stimulate osteoclast differentiation *in vitro* in both mouse- and human-derived cells, *in vivo* mouse models suggest a role in hypoxia-induced bone loss. Our goal was therefore to study role of GDF15 in bone homeostasis using loss of function approaches as opposed to earlier studies in which GDF15 was exogenously administered or overexpressed.

First, we cultured bone marrow-derived osteoclasts from GDF15-KO mice and wild-type (WT). Using TRAP staining and a bone resorption assay, we found that the absence of GDF15 does not affect normal osteoclast differentiation nor function respectively. Next, we determined bone density of WT and KO mice tibiae using μCT. Similarly, there was no *in vivo* bone density phenotype in steady-state conditions. We also evaluated its putative role in in post-menopausal osteoporosis: we found no difference in bone density between KO and WT mice that underwent ovariectomy.

In sum, contrary to what the literature suggests, GDF15 is not required for a normal bone homeostasis *in vitro* nor *in vivo.* Lack of GDF15 also does not affect bone loss in post-menopausal osteoporosis.

TH249. Changes in dietary iron alter the gut microbiome and disease progression in non-obese diabetic mice

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High iron diets are associated with an increased risk of type 1 diabetes (T1D). This may occur through changes in the gut microbiome. Gut bacteria play a role in immune system development and is critical for the onset of hyperglycemia in the non-obese diabetic (NOD) mouse. Here, we examined if dietary iron affects the gut microbiome and disease progression in NOD mice. Mice were maintained on diets containing different amounts of iron starting at 4 weeks of age and blood glucose was measured weekly. The gut microbiome was studied at 8 and 12 weeks of age. We initially compared 2 standard rodent diets, Labdiet 5P04 (~400 ppm iron) and Teklad 2018 (~200 ppm iron), and showed that disease progressed more rapidly in mice maintained on the higher iron diet (77% vs. 45% hyperglycemic by 25 weeks of age). Using custom diets, that differed only by the iron content, we confirmed that higher iron levels exacerbate disease, with 33%, 60%, and 73% of mice developing hyperglycemia by 25 weeks of age when fed diets containing ~200, ~400 and ~1000 ppm iron, respectively. 16S rRNA sequencing showed significant differences in the gut microbiome. In particular, a loss of bacterial diversity was seen as dietary iron levels increased, and significant differences in bacterial strains were observed at different ages. Overall, these data demonstrate that increased dietary iron can exacerbate NOD disease, possibly through a change in the gut microbiome, and that limiting the intake of iron may delay the onset of hyperglycemia.

TH321. Blimp-1 in adipose resident Tregs controls adipocyte browning and obesity

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Visceral adipose tissue regulatory T cells (VAT Tregs) protect against systemic inflammation and metabolic disease by limiting expansion of pro-inflammatory Th1 cells and M1 macrophages, and by preserving insulin sensitivity and glucose tolerance. Although their basic markers and roles have been studied, less is known about the transcriptional machinery regulating their differentiation and function.

B lymphocyte-induced maturation protein-1 (Blimp-1) is a transcriptional regulator known to be involved in the development, polarization, and maintenance of various immune cells including CD4+ T cells. Using Blimp-1 reporter mice, we discovered that Blimp-1 is constitutively expressed in a subset of VAT Tregs compared to lymphoid Tregs, and that Blimp-1+ VAT Tregs are phenotypically distinct from their Blimp-1- counterparts. Blimp-1 is not required for VAT Treg development, however Treg-specific deletion of Blimp-1 led to unique changes in VAT Treg markers in lean versus obese adipose tissue. In addition, Blimp-1 knockout mice fed high fat diet had fewer adipose-resident NK cells and increased CD8 T cells. Surprisingly, loss of Blimp-1+ Tregs led to less adipose tissue IL-10, increased expression of thermogenic genes, reduced body fat, decreased weight, and improved insulin sensitivity. Furthermore, Treg specific deletion of IL-10 led to reduced body weight. Finally, in humans, subcutaneous adipose IL-10 mRNA correlated positively with BMI. Based on these data, we

hypothesize that Blimp-1+ Treg dependent IL-10 production suppresses adipocyte beiging, and that loss of these cells results in increased thermogenesis, greater weight loss and improved insulin sensitivity in obese mice.

TH438. PD-1-expressing Conventional CD4 T Cells are Impaired in the Visceral Fat of Obese Patients with Dysglycemia and exhibit a Proinflammatory Profile

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Insulin-resistance (IR) is often present in obese patients to varying degrees and culminates in type 2 diabetes (T2D) only in a fraction of obese individuals. Adaptive immune cells, including CD4 T cells, are normally present at the target site of inflammation of obesity, i.e. the visceral adipose tissue (VAT). However, if and how they regulate adipocyte metabolism in humans is still largely unexplored. Here we explored the profile of PD1+ CD4 conventional T cells (Tconv) infiltrating the VAT of obese patients with dysglycemia (OBD) compared to normoglycemic obese (OBND). Shortage of PD1+ CD4 Tconv was evident in the VAT of OBD accompanied by elevated production of TNFα upon in vitro non-specific stimulation. Whole-transcriptome sequencing of VAT-derived PD1+ CD4 Tconv showed upregulation of genes involved in T cell differentiation and immune response, and downregulation of genes related to ER stress and lipid metabolism in OBD individuals. Moreover, elevated frequency of VAT resident CD69+PD1+ CD4 Tconv was associated with weight loss after bariatric surgery. Collectively, these data show that PD1+ CD4 Tconv are reduced and shifted toward a pro-inflammatory profile when glucose tolerance is disrupted in obesity. PD1+ CD4 Tconv have the potential to be endowed with metabolic capacity and to represent a possible target to restore insulin-sensitivity and prevent T2D.

NK Cells and Innate Lymphocytes

F311. Delineating the role of human innate lymphoid cells in kidney homeostasis

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Innate lymphoid cells (ILCs) are a family of innate lymphocytes that orchestrate immune responses in cooperation with other immune and non-immune cells to direct tissue homeostasis and respond to perturbations. NK cells are cytotoxic and produce IFN-g, while other ILCs are non-cytotoxic and produce cytokines and other cellular mediators; ILC1s produce IFN-g, ILC2s produce IL-4, IL-5, IL-9 and/or IL-13 and ILC3s produce IL-22 alone or combined with IL-17 or GM-CSF. Recently, regulatory

ILCs that inhibit immune responses have been discovered in the kidney and elsewhere, while conflicting reports suggest that NK cells can exhibit immunoregulatory functions or contribute to tissue destruction. To delineate the role of human ILCs in kidney homeostasis and map their interactions with other immune and non-immune cells, we performed single-cell RNA sequencing of 20 healthy kidney samples from living kidney donors. Parenchymal populations from glomerular (podocytes, endothelial cells) and tubulointerstitial compartments (Loop of Henle, distal convoluted tubule, principal cells and intercalated cells) are featured on our map. Immune infiltrates include distinct T cells, monocytes, macrophages, B cells, and populations of ILCs. Using computational approaches, we are able to predict cellular interactions between ILC subsets and immune and parenchymal cells at homeostasis. Ongoing studies are functionally characterizing kidney-resident ILCs and validating potential interactions with other immune cells. To date, this is the most comprehensive map of healthy human kidney, providing important insights into the functions of ILCs and other immune cells at homeostasis, and is an important reference to map changes that occur with various kidney pathologies.

F329. Epstein-Barr virus peptides derived from latent proteins prevent NKG2A+ NK cell inhibition

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Epstein–Barr virus (EBV) infects more than 90% of adults worldwide and is associated with several malignancies. Reports suggest that innate immune responses including Natural Killer (NK) cells are critical in host defense to EBV. Previously, we demonstrated that a NK cell subset expressing NKG2A/CD94, an inhibitory receptor that recognizes HLA-E, responds to autologous B cells latently infected with EBV.

In silico analysis generated a peptide library derived from EBV latent cycle proteins (LMPs and EBNAs). A peptide stabilization assay confirmed that a subset of the peptides bound to HLA-E. Degranulation assays, using CD107a, demonstrated that some peptides that bound to HLA-E were able to prevent NKG2A+ NK cell inhibition. Peptides derived from LMPs were associated with NK cell degranulation while peptides from EBNAs inhibited effector function. The LMP1 C-terminal domain (192-386aa) was amplified and sequenced from DNA isolated from whole blood of 79 pediatric transplant recipients. A ninemer corresponding to one peptide (GGDPHLPTL) was found in 82% of patient samples while variations of this peptide were detected in the remaining patients (18%). Two sequence variations (4% of patients) demonstrated comparable stabilization of HLA-E as the original peptide, while three other variations, detected in 14% of patient samples, did not stabilize HLA-E.

We demonstrated that peptides from EBV latent cycle proteins can bind to HLA-E and promote NK cell degranulation. Some transplant recipients have variations in the sequence of the LMP1 protein that may prevent NK-cell mediated killing of EBV-infected cells and increase the risk of developing EBV related diseases.

TH51. Effector Memory T Cells in Psoriatic Arthritis: Th17 Cell Predominance With Both Invariant and Conventional αβ T cell Immune Response

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Effector memory T cell (TEM cells) response in autoimmune diseases are of various T cell subpopulations- Th1, Th2, TH17, NKT, MAIT, etc. In psoriatic arthritis (PsA) contributing role of Th17 cells have been observed and success of IL17/IL-23 targeted therapies further substantiated this. Here we will share our cumulative data on Th17 cells in PsA collected over last 2 decades. PBMCs and synovial fluid mononuclear cells (SFMCs) were collected from age/sex matched with active PsA, rheumatoid arthritis (RA) and osteoarthritis patients (OA,n=50,each). Magnetically sorted CD3+ T cells were isolated from PBMCs and SFMCs; were activated (10^6 cells/ml) with anti-human CD3/CD28 cocktail and cultured in RPMI medium for 5 days. Hi-D FACS studies were performed to: (i) identify activated memory cells (CD3+CD45RO+) T cells (ii) identify phenotypes of IL-17A+ T cells such as αβTCR+, γδ TCR+ T cells, iNKT and MAIT cells. We noticed a marked polyfunctionality in PBMC/SFMC T cells both in PsA and RA. In OA activated TEM cells were < 1%. In PsA SFMC, memory T cells (CD3+CD4+CD45RO+) had IL-17A+ (18+0.5%), IL-22+(7+0.3%) and IL-23R+ (8.5+0.5%) T cells compared to SFMC memory T cells of RA (6.8+0.3%, 2.5+0.5%, 2.1+0.7%, respectively; $p< 0.001$). IL-17A+ TEM phenotypes in SFMC of PsA demonstrated both invariant and conventional αβT cell Immune response: αβTCR+ (79.8+0.9%), γδ TCR+ (1.5+0.2%), iNKT (15.5+1.1%) and MAIT (4.5+0.8%) cells. Majority of the αβTCR+ T cells were CD4+ (>85%) whereas MAIT were predominantly CD8+ (~ 90%). In PsA TEM cells were polyfunctional, skewed towards conventional activated memory αβTh17 cells.

TH189. NK Cell-Mediated Lysis of TIGIT+ Cells and Adenosine Impairment of NK Cell Function

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Natural Killer (NK) cells, being part of the innate lymphoid system, are endowed with cytolytic capabilities that can be harnessed as a potent cancer immunotherapy. These cells express function-inhibiting receptors such as TIGIT (T-cell immunoreceptor with Ig and ITIM domains) and A2aR (adenosine 2a receptor) that cancer cells utilize to evade immune invasion. Tumor infiltrating lymphocytes such as CD8+ T cells and NK cells express high levels of TIGIT. Additionally, adenosine, a product of ATP/NAD hydrolysis, is abundant in the tumor microenvironment, where it exerts suppressive effects on these immune cells via A2aR, leading to impaired activation and proliferation. Antigen-experienced CD8+ T cells isolated from human tumor samples (n=5) express high levels of TIGIT and PD-1; thus, antibodies against these proteins that engage Fc-receptors can result in NK-mediated target cell lysis. AB154, a fully humanized α-TIGIT antibody, blocks TIGIT/CD155 interactions at concentrations < 1nM. AB154 and various Fc variants were tested in antibody-dependent cell cytotoxicity (ADCC) studies. Co-culture of activated CD8+ T cells (expressing high TIGIT levels) and NK cells with the non-fucosylated version of AB154 resulted in a 55% ± 5.9% increase in NK-mediated lysis, relative to the Fc-silent version (n=4). NK-mediated cytotoxicity is significantly impaired upon activation of A2aR (70.4% + 5.9% vs. control; $p< 0.01$). AB928, a selective and potent small-molecule

antagonist, significantly rescued this impairment (n=2; p< 0.01). Targeting multiple immune checkpoint proteins on NK cells can relieve tumor-induced immunosuppressive effects, allowing for efficient lysis of cancer cells. AB154 and AB928 are currently in clinical development.

TH383. Group 1 innate lymphoid cells (ILCs) reduce liver immunopathology by limiting local IL-2 availability

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Group1ILCs (NK cells and ILC1s) are innate lymphoid cells (ILCs) which have a crucial role in bridge the innate and adaptive immune responses. Nevertheless, the precise cellular and molecular mechanisms underlying their capacity to module T cell-mediated liver immunopathology in the context of HBV pathogenesis are not fully defined.

Adoptive transfer of HBV-specific effector $CD8^+$T cells (HBV-T_E) into HBV replication-competent transgenic mice resulted in a 15-fold expansion of ILC1s and a 30-fold expansion of NK cells. Depletion of Group1ILCs considerably increased the number of intrahepatic HBV-T_E and the attending immunopathology, which is consistent with a previously described role of NK cells in regulating T cell responses. To investigate the mechanism underlying this observation, we generated Group1ILC reporter mice and performed intravital microscopy of their livers. We observed that Group1ILCs engage in prolonged, stable interactions with $CD8^+T_E$ cells undergoing hepatocellular antigen recognition; however, we did not find any evidence to support the wide-held belief that Group1ILCs control T cell responses by direct cell killing. Moreover, Group1ILCs that lacked perforin or NKp46 (two molecules that were proposed to mediate NK cell killing of T cells) still restrained liver immunopathology. Rather, we found that in the absence of the a-subunit of the IL-2 receptor, Group1ILCs failed to reduce T cell-mediated liver immunopathology.

Taken together, these results reveal a new potential role for Group1ILCs in controlling liver immunopathology by limiting the local availability of IL-2 and have important implications for the treatment of chronic hepatitis B virus infection.

Stem Cell and Organ Transplantation

F33. CMV Responsive TCR Repertoire Evolution Following Solid Organ Transplant

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Cytomegalovirus (CMV) reactivation decreases allograft survival after solid organ transplantation. CMV establishes lifelong latency in infected hosts. Control of CMV reactivation by immune memory is impaired by graft-protective immunosuppressive treatment. Our preliminary study of CMV-responsive memory CD8 T cells demonstrated that these cells expand over the first year after heart or kidney transplantation, despite no clinically detected CMV viremia. This led to the hypothesis that the function of individual T cells or clones is altered, and potentially less protective, after transplantation. To address this hypothesis, we completed single cell sequencing of paired TCRαβ and a panel of functional genes in CMV-responsive T cells from 6 patients pre- and 3 and 12 months post-transplant. Diversity of CMV-responsive TCR clones decreased during the time course. In the majority of patients, this change in diversity was driven by expansion of a single pre-existing clone, independent of the pre-transplant size of the clone. Several shared CMV-responsive TCR motifs were detected despite partial or complete MHC mismatch, suggesting shared CMV specificity. Across patients, the relative size of a clone correlated with its degree of polyfunctionality, or number of cytotoxic functions expressed. The number of functions expressed per cell also increases over the time course, both within clone and across all CMV-responsive T cells. These findings suggest that the post-transplant expansion of CMV-responsive T cells is directed against the virus and represents an ongoing protective, cytotoxic response.

F156. Imunomodulatory effect of Mesenchymal Stromal Cells in Kidney Transplant Patients in an Autologus and Allogeneic setting.

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Aim

Mesenchymal stromal cells (MSCs) have emerged as a promising approach for solid organ transplantation (SOTx) due to their immunomodulatory properties. In view of this, we studied the effect of bone-marrow-derived MSCs (BM-MSCs) on Immune cell profile of kidney transplant (KTx) patients. MSCs used for infusion were either autologous (auto-MSCs) or allogeneic- from respective kidney donor (allo-MSCs).

Methods

For this study, we recruited 17 patients undergoing KTx with living-related donors. These patients were divided into 3 groups: auto (n=6), allo (n=6) and control (n=5). One patient, each from auto and allo group was lost to follow-up. Auto and allo group patients received an intravenous infusion of 1.5X106 BM-MSCs/kg body weight, one day prior to KTx (D-0) and 30 days after KTx (D-30).

Results

Flow cytometric analysis revealed an increase in B regulatory cells, non-conventional T regulatory cells and a decrease in T effector cells corresponding to alteration in cytokine profile in auto-MSC treated patients.

Conclusion

Ours is the first report to compare the effect of auto-MSCs and allo-MSCs on Immune-cell profile of KTx patients. Notably, our findings imply that auto-MSCs in combination with IS drugs induce regulatory B and T cell subsets, which are known to play crucial role in regulating allo-immune responses.
Our results stress upon the novel trend of change in immune cell subsets that will help us to conduct more targeted clinical trials to eventually improve long-term graft survival by selecting therapies appropriate to immune response involved.

F187. Evaluation of a Novel Conditioning Regimen: Post-Transplant Cyclophosphamide, Sirolimus and CTLA4-Ig in MHC-Mismatched Murine Allotransplantation

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) offers a curative option for sickle cell disease patients. Post-transplantation cyclophosphamide (PT-Cy) and sirolimus (Sir) synergistically induce stable mixed chimerism. Further, Sir and CTLA-4 Immunoglobulin (Ig) promote immune tolerance and allograft survival. Our prior conditioning regimen consists of PT-Cy (200mg/kg), and Sir (3mg/kg). Here, in major histocompatibility complex-mismatched allo-HSCT model (Balb/C: $H2K^d$ to B6: $H2K^b$), we attempted to lower the dose of Cy, and added CTLA-4 Ig with or without the T cell depleting agent, anti-Thy1.2. Low dose PT-Cy (50 mg/kg) with Sir and CTLA-4 Ig (2.5mg/kg), referred to as Tri-drug, induced similar donor chimerism levels compared to high dose PT-Cy. Conversely, mice that received low dose PT-Cy and Sir rejected their grafts. In addition, donor chimerism did not increase with Tri-drug and T cell depletion. Compared to low dose PT-Cy and Sir alone, Tri-drug significantly reduced the frequencies of $H2K^b$-specific IFN-g producing $CD4^+$ and $CD8^+$ T cells and NK cells, diminished the frequencies of B cells and DCs, and increased the frequencies of Tregs. Besides, Tri-drug treated mice splenocytes showed no proliferation upon restimulation with Balb/c mice stimulators in an *in vitro* mixed lymphocyte reaction, suggesting donor unresponsiveness. Our data indicate that low dose PT-Cy, Sir and CTLA-4 Ig induce stable mixed chimerism, and immune tolerance is mediated by higher Tregs and lower inflammatory T cells, NK cells and DCs. CTLA-4 Ig may be considered to reduce graft rejection in patients with SCD who undergo HSCT and receive PT-Cy and sirolimus.

F219. A Human Ex Vivo Model Of Antibody Mediated Rejection In The Kidney Using Normothermic Machine Perfusion with HLA & ABO Antibodies

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Background

Currently, no human experimental model of antibody mediated rejection (AMR) exists. Such a model would be vital to investigate therapeutic interventions in organ transplantation. The aim of our work was to establish the first human kidney model of AMR using ex-vivo normothermic perfusion (EVNP).

Methods

We developed AMR models using HLA incompatible (HLAi) and ABO antibodies (ABOi) in 10 discarded human kidneys (including 3 pairs). All kidneys underwent standard pre-experimental EVNP stabilization. For each experiment, we injected fresh frozen plasma as a source of complement/coagulation factors & either 600 micrograms of W6/32 anti-class 1 HLA antibody (for HLAi model) or (pre-tested) high blood group antibody-titre FFP alone (for ABOi model). EVNP renal blood flow (RBFi ml/min/100g), C3 desArg and biopsies were taken. Endpoints included: haemodynamic changes, thrombosis and complement fixation.

Results

A total of 3 HLAi, 5 controls and 2 ABOi experiments were performed. Between 30-45 minutes post-antibody injection, all 3 HLAi and 1 high-titre ABOi kidney showed catastrophic collapse in RBFi (change ranging from 0 to -122 ml/min/100g and 0 to -93.6 ml/min/100g for HLAi and ABOi kidneys, respectively) compared to controls. All HLAi kidneys fixed C4d. Histological microvascular thrombi were

present in one HLAi and one ABOi kidney. There was evidence of complement activation: mean C3 desArg % change from baseline = +115 % HLAi; +199 % controls; +103% ABOi, kidneys.

Conclusion

We have established the first human ex-vivo model of AMR using warm machine perfusion. This model offers a unique platform for testing localised cytoprotective agents.

F221. Mismatched Family Donor Haematopoietic Stem Cell Transplantation (HSCT) in Primary Immune Deficiency (PID)–Single Centre Experience.

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Background-

PID patients receiving HSCT from mismatched family donor (MMFD) are at risk for graft-versus-host disease (GVHD), graft rejection, and progressive pre-existing infection. We report our recent experience with these HSCTs.

Methods

HSCTs were performed from January 2018 to August 2019. Retrospective chart review and summary statistics were conducted for primary diagnosis, conditioning, engraftment kinetics, chimerism, immune reconstitution, and clinical outcome.

Results

Five children with PID had HSCT from MMFD. Primary diagnosis was X-linked severe combined immune deficiency due to IL2RG mutation in 2, Hyper IgM syndrome in 1, X-linked chronic granulomatous disease in 1, and SCID phenotype with ORAI1 mutation in 1. Median age was 2 years (range 1-19 yrs). Fludarabine based reduced toxicity conditioning was used in all children. Selective T cell depletion using TCRαβ and CD45RA depletion was done in 3 children and unmanipulated stem cells infused in 2. All engrafted with full donor chimerism. Adenovirus reactivation was seen in 2 children and cytomegalovirus in another 2. Three children had pre-existing BCGitis with psedo-progression in 1 post-transplant. Immune reconstitution at D+90 showed median CD4 count of 162 (range 110-251) and CD8 count of 93 (range 79-1256). Transient grade 3 skin GVHD was noted in 1 child and none had chronic GVHD. All children are alive at median of 8 months (range 5-20 months) post-transplant without any major complication.

Conclusion-

Contemporary MMFD transplants showed excellent result with minimal conventional complications in this small cohort of children with PID.

F265. Unsupervised Analysis of the Alloimmune Response using Single-Cell Mass Cytometry

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Alloimmune responses in acute solid organ transplant rejection are complex, involving multiple interacting cell types. Understanding the phenotype of these leukocytes has been limited by technology that lack the capacity to resolve high-dimensional interactions. Here, we utilize single-cell mass cytometry using 44 metal-conjugated antibodies to conduct multi-parametric, high-resolution analysis of the alloimmune response in early acute rejection. We profiled a well-established murine cardiac allotransplant model and a novel murine vascularized composite allotransplant (VCA) model across multiple time-points post-transplantation. In allogeneic transplant recipients, there is marked phenotypic heterogeneity within the effector $CD8^+$ T cell and NK cell populations that expand during acute rejection. Three distinct T cell and NK cell subsets were identified and characterized as specifically responding to alloantigen. Contrastingly, the only effector $CD4^+$ T cells associated with rejection are uniquely $CD40L^+$. The myeloid compartment has a significant increase in $Ly6C^{hi}$ inflammatory monocytes. Finally, we report a relatively conserved alloimmune profile between heart and VCA transplants; however, effector $CD8^+$ T cells arise earlier (day 3) in VCA. Using CyTOF to deconvolve and visualize complex systems greatly enhances the capacity to capture both global and subtle changes across multiple cell types and phenotypic markers. This systems-level approach lays the foundation for a comprehensive roadmap of the alloimmune response.

F281. T-cell Clonal Dynamics Determined by High Resolution TCR-β Sequencing in Recipients of Allogeneic Hematopoietic Cell Transplantation

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Delayed reconstitution of the immune system after allogeneic hematopoietic cell transplantation (HCT) is a long-recognized complication. Specifically, loss of T-cell diversity has been implicated in infectious complications, graft-versus-host disease (GVHD), and disease relapse. We performed serial high-resolution next generation sequencing using the immunoSEQ® Assay (Adaptive Biotechnologies, Seattle, WA) to characterize the TCRβ locus in 99 HCT recipients in the first 3 months after transplant. Transplant donor type included unrelated (n=57) and related (n=42) donors. Conditioning regimen intensity included reduced intensity (n=55) and myeloablative (n=44). We measured T-cell fraction, clonality, and richness in the donor and at days +15, +30, +50 and +100 post-transplant in the recipient, and correlated metrics to clinical variables. In agreement with prior studies, we found that although

absolute T-cell numbers recover relatively quickly after transplant, repertoire diversity remains diminished. Restricted diversity was associated with conditioning intensity, use of ATG, and donor type. Increased T-cell clonal expansion at Day +30 compared to the donor sample was associated with the incidence of acute GVHD (HR=1.11, $p=5x10^{-5}$). Even after exclusion of the twelve patients who had experienced acute GVHD by day +30, the association between acute GVHD and clonal expansion persisted (HR=1.098, p=0.041), indicating that clonal expansion preceded the development of acute GVHD. Our results indicate the importance of early post-transplant sampling and highlight T-cell clonal expansion as a potential novel biomarker for GVHD which warrants further study.

F297. Three-year patient and kidney graft outcomes using Expanded Criteria Donors (ECDs) or high UKKDRI criteria.

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We analyzed all adult deceased donor (DD) kidney transplants performed between 2012-2013 over 3 years, comparing outcomes using standard criteria donors (SCD) with ECDs as well as using UKKDRI.

Of the 257 DD kidneys transplanted, 131(51%) were SCD kidneys and 126(49%) ECD. DGF occurred in 107 (41.6%) recipients. There was no significant difference between DGF in recipients who received SCD and ECD kidneys. 37% of SCD kidney recipients experienced DGF, whereas this was the case in 47% of ECD recipients. There was no difference between donor HTN (p=0.488), DM (p=0.533) and graft outcomes at 3 years. 10.3% SCD and 13.5% of ECD transplants failed after 3 years. Graft survival was 79.4% in SCD recipients and 78.4% in ECD recipients at 3 years. There was no difference between graft (p=0.321) and patient survival (p=0.371) between SCD and ECD recipients 3 years. The median UKKDRI for standard risk kidneys was 1.021 (IQR = 0.256) and 1.593 (IQR = 0.504) for high risk kidneys. Kaplan Meier analysis showed no difference between high risk and standard risk kidneys in terms of patient (log rank p=0.483) and graft survival (log rank p=0.776). There was no significant difference between the occurrence of DGF in standard and high-risk kidneys (p=0.455).

There was no significant difference between patient and graft survival 3 years post transplantation in standard and high-risk kidney recipients using UKKDRI. 3-year graft and patient survival was not significantly different between SCD and ECD kidney recipients. This can aid consenting of patients receiving ECD/high risk kidneys.

F433. Impact of neutrophil extracellular traps (NETs) in primary graft dysfunction following human lung transplantation

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Background/Purpose: Primary graft dysfunction (PGD), the major complication associated with lung transplantation within the peri-operative period, is characterized by prominent graft vascular resistance and tissue edema within 72hrs. Among recipients, 15% of them develop the most severe form of PGD (Grade 3; PGD3), leading to 50% of mortality. Recent animal studies suggest that neutrophils can contribute to the inflammatory process through the release of neutrophil extracellular traps (NETs). NETs are composed of DNA filaments decorated with granular proteins, which can contribute to vascular occlusion associated with PGD. Therefore, our main objective is to correlate NETosis markers with recipient clinical outcomes.

Methods: Clinical data and blood samples were collected from donors and recipients (n=32) pre-, intra- and post-operatively (up to 72hrs). Inflammatory biomarkers inducing NETs' synthesis (CRP, IL-6, IL-8) and specific biomarkers of NETs (myeloperoxidase [MPO], MPO-DNA complexes) were quantified by ELISA. When available, histology and immunochemistry techniques were used on donor lung samples collected before transplantation to evaluate the presence of activated neutrophils and NETs.

Results: In lungs from which PGD3 developed within recipients, there is a marked increase of vascular occlusion composed of activated neutrophils and NETs before transplantation. Also, in donors and recipients pre- and intra-operatively, circulating levels of inflammatory (CRP, IL-6, IL-8) and NETosis markers (MPO, MPO-DNA) were up to 4-fold higher in the PGD3 recipients, compared to non-PGD3.

Conclusion: Elevation of these NETosis biomarkers might serve to elaborate an algorithm that would help to better delineate the recipients at risk of developing severe PGD.

TH21. Imatinib controls antibody mediated kidney rejection: Case Report

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Three years after deceased donor kidney transplant a 40yo AA male developed acute cellular rejection when unable to obtain immunosuppressants. Creatinine increased from 1.2 to 1.7; donor specific antibodies (DSAs) became mildly elevated (data below). Simultaneously the patient was diagnosed with chronic myelogenous leukemia (CML). Patient's immunosuppressants were restarted for rejection and imatinib was initiated for CML. Four months later creatinine returned to baseline and white count normalized. However, DSAs increased and in vitro cytotoxicity assay indicated patient's serum lysed donor cells. A second kidney biopsy showed diffuse C4d staining but no inflammation. Low dose IVIG monthly is given to reduce DSA but DR53 titre remains high. Over one year later, creatinine remains at

baseline with positive DSAs. Imatinib has been well tolerated with tacrolimus, mycophenolate and prednisone. The major side effect has been renal magnesium wasting.

Donor specific antibodies levels and clinical history:

Date	Creatinine	Wbc	DR04	DR53	DQ07	Event/Medications
2017	1.2	6000	0	0	0	
Oct 2018	1.7	200,000	1000	2100	4000	Bx 1: Mild ACR. Imatinib, FK, MMF, pred
Dec 2018	1.2	3100				
Feb 2019	1.2	3850	0	24,700	12,800	Bx 2: C4d+, no inflammation. Imatinib, FK, MMF, pred
Dec 2019	1.2	6500	0	24,400	0	

Conclusions: We hypothesize that imatinib, via its tyrosine kinase inhibitor activities, is controlling rejection, possibly by action on lymphocyte associated kinase. The concept of inhibiting antibody-initiated immunity by suppressing cellular response warrants further thought about DSA mechanisms of injury. Imatinib may have a role in antibody-mediated rejection refractory to other therapies.

TH35. Multi-biomarker Integration Enables a Powerful Classification of Long-term Survivors with Normal Allograft Function after Lung Transplantation

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Background: TOLUNG project focuses on discovering biomarkers at clinical, transcriptomic, and immunologic levels in order to characterize long-term survivors with good allograft function after 10 years (LTS) from lung transplantation (LT) with reference to chronic lung allograft dysfunction patients (CLAD). Previously, mRNA and miRNA classifiers were built and allowed a correct classification of LTS patients. Therefore, the objective of the whole TOLUNG project was to assess whether multi-biomarker integration further increases the accuracy of the discrimination of LTS patients from CLAD patients.

Methods: The most relevant features from 4 independent data-blocks (clinical variables, leukocyte subpopulations, mRNA and miRNA) collected simultaneously from 59 bilateral lung transplant recipients (30 LTS, 29 CLAD) were integrated by the DIABLO method. The 4 most relevant clinical variables and the 8 leukocyte subsets which significantly differed between LTS and CLAD groups were used. Furthermore, 21 mRNAs and 4 mature miRNAs were selected from previous individual classifiers built by machine learning (ML) methodology from microarray data which, in turn, were validated by RT-qPCR.

Results: Multi-biomarker integration significantly improved the accuracy of LT patient classification compared with both previous individual mRNA and miRNA ML classifiers, yielding an Area Under the Curve of 0.86 (IC 95% 0.69-1.0).

Conclusion: Integration of 4 biomarker-data blocks enabled 80% correct classification of LT patients. These results can serve to facilitate unsupervised patient classification and after a validation phase, may have utility as a medical tool for safe immunosuppression minimization in LT population.

TH53. Alpha-beta T-cell/CD19 B-cell depleted haploidentical stem cell transplantation: a new platform for curing rare and complex genetic disorders

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Allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-matched donor has been extensively used to treat patients with genetic disorders. However, about 40% of these patients need alternative donors. HLA-haploidentical HSCT after αβ T-cell/CD19 B-cell depletion (αβ haplo-HSCT) has been shown to be effective in curing up to 90% of children with a variety of non-malignant disorders. However, a scarcity of data are available for patients with rare and complex genetic diseases, including Tregopathies, an emerging new class of primary immunodeficiencies.

Between 05/2018 and 08/2019, five patients with complex monogenic disorders were referred to Lucile Packard Children's for HSCT including: a 23-year-old with Fanconi Anemia, two with Immune dysregulation-polyendocrinopathy-enteropathy, X-linked (IPEX), and two with Schimke-Immuno-

Osseous Dysplasia (SIOD). Remarkably, all these patients were previously considered not eligible for a HSCT, and one of the IPEX patients was in palliative care. At a median follow-up of 270 days (range 39-550), all patients are alive and disease-free. Five months after αβ haplo-HSCT and with 100% of the circulating T cells being donor derived, the first SIOD patient received a living kidney transplant from the stem cell donor (mother) using minimal immunosuppression with tacrolimus and steroid. Four weeks after the kidney transplant all immunosuppression was stopped, and the patient remains off immunosuppression 22 weeks post-kidney transplantation. Our data provide a strong rationale for expanding the use of αβ haplo-HSCT to diseases not previously candidate for allogeneic HSCT, to patients in very poor clinical condition and to patients requiring both HSCT and solid organ transplant.

TH104. HLA Matching (HM) Score in Renal Transplantation

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Introduction: The conventional HLA matching method with MM score introduces a big challenge of uncertainties in identifying the matching antigens and the loci. A mathematical method has been developed to quantify the degree of HLA matching that can be used in the comparison of any number of loci. This method can be applied directly with both low resolution and high resolution HLA data.

Materials and Method: A numerical method analogous to the positional number system has been developed to quantify the degree of HLA match between the recipient and the donor. Two terms, namely Antigen/Allele Matching Score (AMS) and HLA Matching Score (HM score) have been introduced and defined. AMS has been defined as the sum of locus weighted score assigned to each antigen match when comparing the HLA of the recipient and donor with a matching hierarchy of DR >B >A. HLA Matching Score is defined as the ratio between the AMS obtained and the maximum possible value of AMS.

Result: There are 27 and 216 discrete HM scores observed between 0 and 1 in the serologic and allelic HLA data comparisons respectively. Donor with the highest HM score is the best match.

Conclusion: HLA matching score is quantitative, with no overlap of scores, and is ideal in computerized donor selection method (KAS of UNOS/OPTN). Graft survival study done with HM score generates single curve for every HLA matching combination. This method overcomes all the problems that are found in the conventional MM scoring method.

TH105. Graft Survival Studies with HLA Matching Score

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Introduction: All the Graft Survival studies are done with MM scores introduce challenges in the analysis, that are done by comparing the mismatched in HLA- DR, B and A antigens. Graft Survival Studies done with Mismatch scores have many inherent concerns. Most important being the overlap of

the MM scores, with the inability to represent very matching situation even in the three loci comparison. Degree of overlap increases many folds when matches in more number of loci are performed with the MM scores. Therefore the outcome for every single matching situation remains unclear with MM scores.

Material and Method: This Graft survival studies has been done with a quantitative score named as HLA Matching HM (HM) score that is unique for every single HLA matching combination. A survival study done with MisMatch (MM) using from other another published study has been used in this study. All the possible Kaplan- Meier curves with HM score are generated

Result: There are 27 different survival curves generated between zero and 100 percent of HLA matching score in Renal Transplantation. There are 1,3,6,7,6,3 and 1 number of survival curves are seen for the 0,1,2,3,4,5 and 6 MM scores respectively. It is also possible to do the survival studies in comparing matches in DP, DQ loci.

Conclusion: Graft survival studies are possible for every single HLA matching situation using the HLA Matching Score. Survival studies done with HLA Matching scores could provide more valuable information regarding the significance of antigen matches in various loci.

TH306. Critical roles of TIGIT+ human equivalent of B10 Bregs in immune regulation

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Regulatory B cells (Bregs) play an important role in immune suppression. However, phenotypic and functional characteristics of human Bregs remain elusive. Here, we report that surface TIGIT$^+$ human Bregs play critical roles in immune regulation. Using data from B cell surface array and RNA-seq, we identified two major TIGIT$^+$ human Breg subsets, CD24^{hi}CD27$^+$CD39^{hi}IgD$^+$IgM^{hi}CD1c^{hi} marginal zone (MZ)-like Bregs and CD24^{hi}CD27$^+$CD39$^+$IgD$^-$IgM$^+$CD1c$^+$ memory Bregs, that are originated from CD24^{hi}CD27$^+$ human equivalent of B10 cells. Similar to immature transitional B cells (TBs), they were able to suppress T cell responses via the action of IL-10 and surface PD-L1. However, they were also highly capable of suppressing T cell responses by expressing surface TIGIT and granzyme B as well as TGFb1. In addition, we also demonstrated that TIGIT$^+$ Bregs can control immune responses by altering dendritic cell functions. We further found that liver allograft recipients with donor-specific alloantibody (DSA) had lower frequency of TIGIT$^+$ Bregs than those without DSA. Moreover, the frequency of TIGIT$^+$ Bregs correlated with that of CD25$^+$CD127^{lo} Tregs, while it inversely correlated with the frequency of follicular helper T cells. We thus concluded that TIGIT$^+$ Bregs play essential roles in immune regulation.

TH313. Single Cell Immune Profiling in Human Intestinal Allografts Reveals Heterogeneity and Alloreactivity of Recipient Resident Memory T cells in Association with Graft Outcomes

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Host-versus-graft (HvG) T cell clones (defined by high throughput sequencing of alloreactive clones from pre-transplant mixed lymphocyte reactions) are enriched in the graft mucosa during early rejection after human intestinal transplantation and persist despite rejection resolution. We integrated clonotype, alloreactivity and gene expression profiles of allograft recipient T cells at the single cell level to assess the functional phenotype of alloreactive T cells in association with graft outcomes.

Recipient T cells sorted from graft mucosa of three quiescent and one chronically rejecting allografts and a deceased donor intestine shared four populations: multifunctional ($IL17A^+$, $IL22^+$, TNF^+) resident memory T cells (TRMs) ($CD69^+$, $ITGAE^+$, $CXCR6^+$, $PRDM1^+$) that were enriched for HvG clones; nonTRMs ($CCR7^+$, $KLF2^+$); cytotoxic CD8 and γδ TRMs ($GZMA^+$, $GNLY^+$); and regulatory T cells. Cytotoxic ($GZMB^+$, $PRF1^+$) effector T cells (Teff) that were enriched for CD8 HvG clones were identified only in the graft with chronic rejection. T follicular helper (Tfh) cells lacking a TRM signature that were enriched for CD4 HvG clones were identified in one quiescent graft with mucosal lymphoid follicles. Partial tolerance of circulating recipient T cells to donor antigens developed post-transplant. Tolerance of circulating pre-existing HvG clones and a paucity of *de novo* HvG clones in quiescent allografts suggest a tolerogenic effect of the graft.

We have demonstrated heterogeneous contributions of pre-existing HvG-reactive T cells to TRM and Tfh compartments in quiescent allograft recipients and evidence for donor-specific tolerance. Chronic rejection is associated with a cytotoxic Teff phenotype that were enriched for CD8 HvG clones.

TH334. Hedgehog Signaling Selectively Assembles NLRP3 Inflammasomes in ZFYVE21hi T Peripheral Helper Cells During Ischemia Reperfusion Injury

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De novo donor specific antibody (dnDSA) are anti-MHC alloantibodies that bind to endothelial cells (ECs) of solid organ allografts to promote ischemic complications and late allograft loss. Ischemia reperfusion injury (IRI) is a pathological process that predisposes to the development of dnDSA, but underlying mechanism(s) are unknown. We identified a cell population expressing ZFYVE21, a novel Rab5 effector, as a mediator of IRI-induced dnDSA. ECs subjected to IRI become Hedgehog ligand-producing cells in a complement-dependent manner *in vitro* and *in vivo.* EC-derived Hedgehog ligands induced ZFYVE21 in CD4+CD45RO+PD-1hiCCR2+CXCR5- T cells, a cell population displaying a cell surface marker, transcriptional, and effector phenotype compatible with recently described T peripheral helper (TPH) cells. In these cells, ZFYVE21 elicited Akt-dependent assembly of an NRLP3 inflammasome to elicit IL-18-mediated expansion of an IL-18R1+ subset of this cell population to promote dnDSA. In a humanized mouse model, we used pharmacologic and genetic approaches to support a role for Hedgehog-induced inflammasomes and ZFYVE21 in promoting dnDSA-related vasculopathy. Patients with delayed graft function (DGF), a clinical manifestation of IRI, showed Hedgehog ligand production in complement-bound ECs, and demonstrated higher levels of ZFYVE21 and cleaved caspase-1 relative to controls. Sera from DGF patients containing high levels of Hedgehog ligands moreover potentiated ZFYVE21, IL-18, and TPH cell activation, and these effects were reversed with vismodegib, an FDA-approved Hedgehog signal antagonist. Hedgehog ligands released by endothelial cells following IRI expand alloimmune ZFYVE21^{hi} T peripheral helper cells to promote dnDSA and its related vascular pathologies.

TH341. Immune Mechanisms of Disparate Liver Transplant Outcomes in Female Hispanics with Nonalcoholic Steatohepatitis

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Obesity and metabolic syndrome have become an epidemic with Hispanics bearing a disproportionate burden. Non-alcoholic steatohepatitis (NASH) is a severe immune-mediated stage of non-alcoholic fatty liver disease, the most common liver pathology associated with obesity/metabolic syndrome, and can progress to end-stage liver disease requiring orthotopic liver transplant (OLT) for survival. This study investigates underlying immune mechanisms of disparity experienced by Hispanics undergoing OLT for NASH. We enrolled 128 OLT recipients in our IRB-approved study, 25 of which presented with NASH as the primary etiology for OLT (20%). We investigated evolving patient immune status via longitudinal HLA single-antigen bead testing, histopathology, 38-plex cytokines, and immune cell phenotypes via flow cytometry/ELISA. 6/9 IRI+ patients with NASH were self-reported Hispanic (67%), and all 6 (100%) were pre-sensitized to HLA antigens. Remarkably, 84% of NASH patients had significant macrovesicular steatosis deposited into donor hepatocytes, and 52% had hepatocellular ballooning by 2 hrs post-reperfusion, which included 100% of females despite the overall cohort being predominantly male. Blood from NASH patients produced a pro-inflammatory, pro-apoptotic macrophage phenotype with reduced CD14/CD68/CD66a/TIM-3 and increased CD16/HLA-DR along with significantly elevated circulating levels of IL-17A/IP-10/MCP-1. Female Hispanic NASH patients required more for-cause biopsies and had worse outcomes, including ACR/AMR/Death. Taken together, female Hispanics with

NASH in our OLT cohort disproportionately suffered from immune-mediated OLT complications leading to worsened outcomes within the first year post-transplant. As such, reduction of IRI and other immunological risk factors should be considered in Hispanics with NASH requiring OLT, particularly females.

TH346. Impact of hematopoietic stem and progenitor cell (HSPC) composition on lymphohematopoietic recovery after αβ-T-cell/CD19 B-cell depleted haploidentical transplantation.

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Previously identified factors that influence hematopoietic recovery have focused on the enumeration of bulk $CD34^+$ and of mature effector cells, such as αβ/γδ T and NK cells. Information about HSPC graft composition and its relationship with clinical outcomes are lacking. We hypothesized that variations in HSPC subpopulations, frequency and number may contribute to differences in lymphohematopoietic recovery and, therefore, clinical outcome.
Our cohort includes six αβ T-cell/CD19 B-cell depleted haploidentical (αβhaplo)-HSCT recipients: 3 patients with robust hematopoietic recovery (Group 1), and 3 patients who experienced mild cytopenia after Day 60 (Group 2). All patients were transplanted for acute leukemia and had received a myeloablative TBI-based conditioning regimen.We correlated the HSPC composition of the graft infused at Day 0 and of the bone marrow (BM) evaluated at Days 30, 60, and 90 after αβhaplo-HSCT, with the peripheral blood (PB) immune recovery at the same time points.
Our preliminary data indicate that even using a consistent method of graft manipulation, HSPC graft composition is heterogeneous. We found a significative correlation between the GMP and CMP subpopulations in BM and PB myeloid counts, while none or a weak correlation existed between the CLP and HSC subpopulations with lymphoid and hematopoietic counts- with the exception of platelets and γδ T-cells in Group 1. The evaluation of a larger number of patients with longer follow up after HSCT is required. Such analyses will be instrumental not only for prediction of clinical outcome, but also for optimization of novel graft engineering strategies.

TH347. Newborn T Cells and Their Progenitors Represent a Unique State Along a Progressive Transition from Fetal to Adult Immunity

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Whereas the human fetal immune system is poised to generate immune tolerance and suppress inflammation *in utero*, an adult-like immune system must emerge to orchestrate anti-pathogen immune responses in post-natal life. It has been posited that cells of the adult immune system arise as a discrete ontological wave, or layer, of hematopoietic stem-progenitor cells (HSPCs) and their progeny, but evidence supporting this model in humans has been inconclusive. We combined bulk and single-cell transcriptional profiling of naïve T cells, classical monocytes, and HSPCs from fetal, perinatal, and adult developmental stages with novel bioinformatics and machine learning techniques to demonstrate that the fetal-to-adult transition occurs progressively along a continuum of maturity – with a substantial degree of interindividual variation in transition progress by the time of birth – rather than via a transition between discrete waves. We then investigated both shared and unique pathways that differentiate umbilical cord blood (UCB) naïve T cells from their fetal and adult counterparts. While immune activation pathways were upregulated in adult with respect to both fetal and UCB naive T cells, Wnt and Notch signaling pathways, which play known roles in T cell differentiation, were among those enriched within UCB naïve T cells with respect to adult naïve T cells. These findings have important implications in the design of strategies for vaccination and prophylaxis against infection in the newborn, as well as for umbilical cord blood transplant.

TH349. Contribution of Disulfide-HMGB1 Released During Ischemia-Reperfusion Injury to the Development of Alloimmunity Following Liver Transplantation

Allyson Terry[1], Rebecca Sosa[1], Maura Rossetti[2], Jessica Nevarez-Mejia[2], Bita Naini[2], Fady Kaldas[2], Victoria Groysberg[2], Stephanie Younan[2], Ronald Busuttil[2], David Gjertson[2], Jerzy Kupiec-Weglinski[2] and Elaine Reed[1]

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Ischemia-reperfusion injury (IRI) during liver transplantation (LT) is a pro-inflammatory response linked to increased post-LT alloimmunity, potentially through post-LT innate-adaptive cross-talk. We previously showed that post-reperfusion portal vein blood (LF) from IRI+ LT patients activates TLR4 and induces pro-inflammatory macrophages and that disulfide HMBG1 (diS-HMGB1), whose receptors include TLR4, is increased in IRI+ patients. This study aimed to determine the contribution of diS-HMGB1-TLR4 signaling to the IRI+ polarized macrophage phenotype. Third-party monocytes were exposed to diS-HMGB1 or LPS with or without TAK-242, a TLR4 inhibitor, for 5 days. CD66a, CD86, HLA-DR, PD-L1, and TIM-3 surface expression was analyzed by flow cytometry and compared to phenotypes induced by LF from LT patients. diS-HMGB1 mirrored the IRI+ LF macrophage phenotype in 4/5 markers (high HLA-DR, CD86; low PD-L1, CD66a). diS-HMGB1 trended with LPS for 1/5 markers (CD66a). High TIM-3 induced by diS-HMGB1 mirrored TIM-3 expression induced by IRI- LF. TAK-242 had a dose-dependent effect on 4/5 markers for the LPS phenotype but only 1/5 markers for the diS-HMGB1 phenotype. Together, decreased PD-L1 and CD66a and increased HLA-DR and CD86 induced by both diS-HMGB1 and IRI+ LF suggests that 1) diS-HMGB1 contributes to the post-LT immune response in IRI+ patients, 2) these macrophages have increased antigen presentation and decreased T cell inhibition capabilities, and 3) the observed diS-HMGB1 phenotype is not completely dependent on

TLR4. This study reveals how diS-HMGB1 released during IRI can increase antigen presentation and adaptive immune activation, providing a potential link between IRI and post-LT alloimmunity in IRI+ patients.

Systems Immunology

F151. Mass Cytometry (CyTOF) and Immune Profiling to study rare immune diseases.

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Recently, exome/genome sequencing and transcriptomics have helped solve many diagnostic odysseys. However, cellular and molecular details remain uncharacterized for many rare and undiagnosed immunological diseases that are often non-Mendelian. We used immune-phenotyping CyTOF to detect outlier cell populations in undiagnosed patients with immunological (n=15) and non-immunological (n=9) phenotypes. In a small subset of these patients, we looked at phospho-signaling in disease samples, collected at different time points. We also assessed 62 circulating cytokines in serum from 30 undiagnosed patients. Using a patient with Schnitzler's syndrome as an example of rare immune-mediated disease, we summarize findings from each assay. We observed increase in CXCL10 (average z-score: 2.5) and CCL2 (average z-score: 2.71) in disease samples at four different time points. Comparing the patient's PBMC samples from three different time points to a healthy, age- and sex-matched control, we found decreases in B cells (Naive and Memory B), CD4 T cells (Effector CD4, Effector Memory CD4) and CD8 T cells (Effector CD8, Effector Memory CD8, Central Memory CD8). Four cell types (Central Memory CD8 T, Central Memory CD4 T, Effector Memory CD4 T and Non-classical Monocytes) from this patient consistently showed higher Stat3 phosphorylation on stimulus with IL6 and six cell types (Effector Memory CD8 T, Classical NK, Alternate NK, Classical Monocytes, Non-classical Monocytes and Myeloid Dendritic Cells) had higher Akt response on stimulation with PMA as compared to minor or no response in healthy cells. We will further discuss the immune-phenotyping results from other patients that had outlier cellular profiles.

F250. Systematic study of T-cell receptor repertoire profiling reveals large methodological biases: lessons from a multicenter study

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Accurate profiling of T-cell receptor (TCR) repertoires is key to monitoring adaptive immunity. We systematically compared TCR sequences obtained with 9 methods applied to aliquots of the same T-cell sample. We observed marked differences in accuracy and intra- and inter- method reproducibility for alpha (TRA) and beta (TRB) TCR chains. Most methods showed lower ability to capture TRA than TRB diversity. Low RNA input generated non-representative repertoires. Results from 5'RACE-PCR methods were consistent among themselves, while differing from the RNA-based multiplex-PCR results. gDNA-based multiplex-PCR methods also differed from each other. Using an in silico meta-repertoire generated from 108 replicates, we found that one gDNA-based method and two non-UMI RNA-based methods were more sensitive than UMI methods in detecting rare clonotypes, despite the better clonotype quantification accuracy of the latter. This study delineates the advantages and limitations of different TCR sequencing methods, which should help the study, diagnosis and treatment of human diseases.

F253. Cell type classification and discovery across diseases, technologies, and tissues enables standardized single-cell readouts in the clinic

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Successful targeting of pathogenic pathways in autoimmune diseases requires a detailed understanding of the underlying cellular and molecular phenotypes. This is why single-cell transcriptomic approaches are increasingly making their way into the clinical science. However, as of yet, there is no standardized analytical infrastructure available to efficiently channel these cutting-edge developments into a rational, data-driven and effective way to identify novel targets and biomarkers within a drug development research setting.

We filled this gap by developing a robust, efficient and scalable AI-driven algorithm which a) distinguishes immune cells from non-immune cells in peripheral tissues b) accurately and consistently maps single-cell identities to a detailed hierarchy of known immunophenotypes; and c) identifies potential novel cell types. Importantly, the algorithm has the capacity to learn from experience, both by incorporating vetted novel cell types and by refining the representations of existing phenotypes as it encounters them in different contexts.

The algorithm allows for a standardized and scalable analytic workflow which can be adapted to the clinical setting. The resulting annotation enables a number of automatable downstream analyses such

as cell-type abundance estimates; differential abundance analysis across patient and control groups; cell-type specific differential gene expression analyses and cross-dataset comparisons; It also enables cross-modal data integration, by facilitating the conversion of single-cell data into pseudobulk profiles that can be incorporated into genomic databases. Overall, this versatile approach converts single-cell data into and an objective readout which can be used for the systematic identification of cellular targets and biomarkers.

F254. Dissecting the role of mononuclear phagocytes in Lupus Nephritis with a systems biology toolkit

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The contribution of monocytes and macrophages (MMs) to lupus nephritis (LN) is an emerging area of scientific and clinical interest. Our recent analysis of single-cell data generated within the Accelerated Medicine Partnership reveals the presence of classical (CD14hCD16l) and non-classical (CD14lCD16h) MMs in the kidneys of both LN patients and healthy kidney donors. The non-classical phenotype is proportionally enriched in LN patients, motivating a deeper characterization of its functional characteristics, and its relationship to peripheral blood monocytes which CD14+CD16-, CD14-CD16+, CD14+CD16+ phenotype.

We present an in-depth characterization of the transcriptional phenotypes of the observed kidney MMs, focusing on differences in their antibody clearance, nucleic acid sensing, interferon signaling properties. We develop an approach linking these to transcriptional and cytokine profiles of MM phenotypes derived from blood of healthy volunteers and SLE patients, both before and after performing disease-relevant perturbations.

Further, we focus on putative differential migratory mechanisms by interrogating single-cell data to link kidney chemokine production to chemokine receptor expression observed on the two types of MMs observed. Finally, we present initial results from assays performed in an organ-on-a-chip disease model consisting of polarized, perfused human renal proximal tubules in co-culture with endothelial tubules. The assays allow us to characterize the cytokine production on the proximal tubule side under disease relevant perturbations, as well as the migratory properties of different MM subtypes in a high-throughput way. In summary, our work contributes to the overall understanding of MM involvement in lupus nephritis.

F257. Mapping targets to diseases – a protein network based approach for target efficacy assessment and disease identification

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It has been reported that 54% of the phase 3 clinical trials with novel therapeutics fail due to insufficient efficacy. To improve drug efficacy, having the right target for the right disease is the key. Signaling pathways or molecular networks are often used to illustrate the underlying mechanism of how a target relates to a disease phenotype. To better understand the molecules involved in target function and phenotypes associated with these molecules, we developed a novel algorithm, ANDIE, to construct the target molecular network and establish connection to disease phenotypes. This in silico tool can systematically map targets to diseases based on integrated information of protein interaction network and human disease phenotype data. Specifically, STRINGdb was used as inputs to construct the target molecular network and human GWAS catalog data was used as human disease phenotype annotation. The performance of ANDIE was assessed using a reference set containing targets with FDA approved indications and a sensitivity of 25.2% and a PPV (positive predictive value) of 29.0% were reported. ANDIE has been successfully applied to multiple drug discovery programs in the immunology therapeutic area. By investigating molecular network, the algorithm provides a systematic view of therapeutic efficacy between a target and a disease and results are used to guide program decisions.

F291. Identifying TCR-blocking HLA-specific antibodies as potential therapeutics in achieving transplant tolerance

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Over the last decades, organ transplantation as a curative therapy for organ failure has made rapid progress. However, the adaptive immune system, such as T cells and antibodies targeting human leukocyte antigens (HLA), termed allo-reactive T cells and allo-antibodies, are the leading cause for graft rejection. The presence of anti-donor HLA Abs is considered a risk factor that disqualifies a particular donor-recipient pair.

However, protective blocking function of allo-antibodies has been suggested, and allo-antibodies have been found in some long-term graft survivors. Therefore, the role of allo-antibodies in transplantation remains unclear and more research is needed to effectively evaluate the underpinning mechanisms of graft rejection.

Here, A*1101-specific monoclonal IgG1 antibodies were generated from a non-immune human library using the Phage-Fab display approach, and the effect of antibodies was investigated on A*1101-restricted syngeneic primary T cells and a T cell line expressing a known TCR specific for A*1101.

We identified an A*1101-specific monoclonal IgG1 antibody with the capacity to block TCR recognition and to decrease T cell activation. Furthermore, this antibody reduces the translocation of transcription factors that are needed for T cell effector function and cytokine production. These findings indicate that

non-pathogenic alloantibodies have the potential to be developed into specific treatments targeting mismatched donor HLA molecules.

F395. A Novel Host Transcript Signature for Distinguishing Bacterial and Viral Infections

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Anti-microbial resistance is one of the biggest issues facing humanity today. A major contributing factor to anti-microbial resistance is inappropriate prescription of antibiotics due to inaccurate diagnosis of the type of infection. Existing pathogen-based diagnostics are insufficient to prevent the over-prescription of antibiotics due to several limitations. A growing body of work from us and others has shown that host-based gene expression is a sensitive and specific biomarker for diagnosis of the presence and type of infection.

Using 3,731 samples across 63 datasets, we show that host response-based diagnostic signatures identified using only samples from the US or Western Europe do not distinguish intracellular bacterial infections from viral infections with clinically useful accuracy, and are not generalizable to regions where intracellular bacterial infections are more prevalent. We hypothesized that including intracellular bacterial infections would identify a generalizable host signature for distinguishing bacterial and viral infections. Using a novel statistical framework, we integrated 4,223 samples from 20 countries across 69 independent datasets that represented biological, clinical, and technical heterogeneity observed in the real-world patient population. We identified an 8-gene Bacterial-or-Viral Infection (BoVI) signature that accurately distinguished extracellular and intracellular bacterial infections from viral infections. We validated the accuracy of this signature in two prospective cohorts from two countries using RT-PCR. Importantly, the BoVI signature had 90% sensitivity and 89% specificity in prospective cohorts, which met the target product profile described by the WHO and FIND for a diagnostic test to reduce antibiotic over-prescription, supporting further validation of BoVI in larger cohorts.

F396. Multimodal Analysis of Delayed Blood Processing Shows Wide Impacts on ex vivo Biology

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Multi-omic profiling of human peripheral blood is increasingly utilized to identify biomarkers and pathophysiologic mechanisms of disease. The importance of these platforms in clinical and translational studies led us to investigate the impact of delayed blood processing on the numbers and state of peripheral blood mononuclear cells (PBMC) and on the plasma proteome. To comprehensively measure this impact, we performed high-parameter flow cytometry, targeted gene expression (>500 genes), single-cell RNA sequencing (>500,000 cells) and highly multiplexed proteomics (>1100

proteins) on ten patients at baseline and four or five delayed timepoints. Similar to previous studies, we show minimal effects of delayed processing on the numbers and general phenotype of PBMCs up to 18 hours. In contrast, profound changes in the single-cell transcriptome and composition of the plasma proteome become evident as early as 4 hours after blood draw. These reflect patterns of cellular stress signaling across diverse cell types that lead to progressive distancing of the gene expression state and plasma proteome from native in situ biology. Differences accumulating during an overnight rest (18 hours) would readily mask relevant biologic variance related to many underlying disease states.

F422. Sample-specific Predictive Models of Single-Cell Resolution Abundances of Histone Post-Translational Modifications Reveal Pairs with Highly Conserved Associations in Human Immune Cells

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Histone post-translational modifications (HPTMs) play a crucial role in the development and function of immune cells, and pathogenesis of diseases, including cancer and autoimmune disorders. However, a systematic characterization of HPTMs at a single-cell resolution and their variations across samples has not been possible so far as most of the existing data are obtained from a bulk population of cells and measured only a few HPTMs. We have described EpiTOF, a mass cytometry-based technique, to measure total abundance of 38 HPTMs at a single-cell resolution. Using data from EpiTOF experiments on more than 150 healthy subjects and more than 50 million immune cells, we trained non-linear, sample-specific, single-cell resolution predictive models of HPTMs that predict one HPTM using the other HPTMs as predictors. To this end, we adapted a deep learning algorithm, neural process. We found that the abundance of several HPTMs can be accurately predicted from abundances of other HPTMs. Using a perturbation analysis, we inferred several novel associations between different types of modifications on different histones. Furthermore, we identified 4 HPTMs that together show strong associations with most other HPTMs. Leveraging our large and heterogeneous sample pool, we quantified the variations in these HPTM associations. We found that most HPTM associations are highly conserved. Overall, our data and models provide characterization of HPTMs in the healthy immune system that is robust across all ages and both sexes in hitherto unprecedented details.

F449. How will Multiple Sclerosis Impact Healthcare Utilization and Macroeconomics in the United States by 2040?

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In a time-series epidemiological design, the current investigation forecasts healthcare utilization and macroeconomics of multiple sclerosis (MS) in the US from 2017-2040. Using the National Inpatient

Sample datasets, MS patients hospitalized in the US between 2001-2016 are identified. Primary outcomes were forecasts/projections up to 2040 for MS healthcare utilization [crude annual MS hospitalization rates; case-fatality; discharge disposition] and macroeconomics [costs; hospital stay]. Forecasting estimates are computed from **Autoregressive Integrated Moving Average (ARIMA) modeling,** a novel technique popular among **financial analysts for stock-price prediction,** and compared to the baseline year 2016 as a percentage change in primary measures. In 2030 and 2040, hospitalization rates for MS will increase by approximately 32% and 55%, respectively from baseline 2016[n= 150,430]. During the same time-period, inpatient mortality will not likely see a major deviation from their current trends[0.32% in 2016] compared to 2030[0.22%] or 2040[0.19%]. However, a higher proportion of inpatients will witness non-routine discharges to rehabilitation in 2030[+43%] and 2040[+67%] compared to 2016[n=9619]. Despite shorter hospital stay [5.41 days in 2016 versus -7% in 2030; -13% in 2040], the cost of care will increase by 17% (+$2,073) in 2030 and 28% (+$3,504) in 2040 compared to US$12333 in 2016, when inflation-adjusted to the 2018-dollar amounts. **By 2040, hospitalization for MS will increase ~over 50% compared to 2016.** From a policymaking perspective, the data serves as an adjunct for judicious resource allocation and planning (e.g. **establishment of ACGME-accredited Neuroimmunology fellowships**) for preserving care and economic viability in neuro healthcare delivery while reducing access-disparities.

TH9. Network Analysis framework for high dimensional Cytometry data to enable systems Immunology

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The immune system works in a coordinated network where immune subsets influence each other to generate optimal immune responses, and imbalance/breaks in network communication causes suboptimal or dysregulated immune response. However, an analysis framework to model immune cell communication is lacking and is an unmet medical need. We have developed an integrated database and analysis framework named as EPIC for High dimensional cytometry data analysis. EPIC is available as a web application (epicimmuneatlas.org). In the current study, we propose a network analysis framework for high dimensional cytometry data to capture the complexity of the immune system. The proposed network analysis framework will also be integrated with EPIC. We used healthy control (HC) data from EPIC database, and acquired CyTOF (Cytometry by Time Of Flight) data from SSc (systemic sclerosis), epilepsy and early rheumatoid arthritis (eRA) patients cohort for analysis. We used correlation network and social network analysis tools to model communication among the immune cells, and to investigate immune responses at the system level. Using our novel analysis approach we found emergence of a modular structure in immune cell network of patients from disease cohorts when compared with HC. Furthermore, we found a decrease in regulatory communication and increase in centralization score, indicating dominance of a few cell subsets in immune cell networks from patients.

We believe our analysis approach has immense translational potential to accurately assess the immune system holistically and could be used in diagnostic and prognostic applications.

TH16. Decentralisation of T cell education during gestational development in human fetuses through post thymic immunological experience

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During early gestation, T lymphopoiesis in the fetal thymus originates from the initial infiltration of thymus-seeding progenitors (TSPs) from the fetal liver or bone marrow, which develops into early thymic progenitors (ETPs) and extends towards a thymic T cell selection process. We hypothesised that by mid gestation, T cell education is decentralised from thymic development through extension of T cell education in peripheral tissue microenvironments. We examined the competency of fetal circulatory T cell landscape with mass cytometry, cross referencing against an age profiled T cell database (EPIC, *Nature Biotech*, accepted). uMAP dimensional reduction of FlowSom clusters reveal distinctive segregation of fetal T cells from later life stages. Despite possessing a dominantly naive profile, the fetal systemic profile displays an assortment of memory T effector phenotypes, indicating a developing but competent T cell landscape during gestation. The origin of these T effector phenotypes is reaffirmed by fetal thymic $CD4^+$ and $CD8^+$ single positive cells. Yet continued numerical and memory expansion of T effector phenotypes in fetal peripheral tissue, indicates a post-thymic phase in T cell education that is driven by microenvironments, particularly in the gut where memory formation is skewed. This spectrum of T effector phenotypes is complemented by a thymic driven infiltration of activated and proliferating T regulatory cells and memory expansion in peripheral fetal tissues. Our findings unveil a competent fetal T cell landscape, where its development originates from the thymus and is reshaped and decentralised by the peripheral environment, and constantly restrained by tolerance mechanisms.

TH32. A robust and interpretable, end-to-end deep learning model for cytometry data

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Cytometry technologies are essential tools for immunology research, providing high-throughput measurements of the immune cells at the single-cell level. Traditional approaches in interpreting and using cytometry measurements include manual or automated gating to identify cell subsets from the cytometry data, providing highly intuitive results but may lead to significant information loss, in that additional details in measured or correlated cell signals might be missed. In this study, we propose and test a deep convolutional neural network for analyzing cytometry data in an end-to-end fashion, allowing direct association between raw cytometry data and the clinical outcome of interest. Using nine large CyTOF studies from the open access ImmPort database, we demonstrated that the deep convolutional neural network model can accurately diagnose the latent cytomegalovirus (CMV) in healthy individuals, even when using highly heterogeneous data from different studies. In addition, we adopted two approaches for interpreting the deep convolutional neural network model and identified potentially novel immune cell subsets significantly associated with latent CMV infection. Finally, we provide a tutorial for creating, training and interpreting the tailored deep learning model for cytometry data using Keras and TensorFlow (immport.org/resources/deeplearning).

TH45. Multiplexed Single-cell Metabolic Profiles Organize the Spectrum of Human Cytotoxic T Cells

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Cellular metabolism regulates immune cell activation, differentiation and effector functions to the extent that its perturbation can augment immune responses. However, the analytical technologies available to study cellular metabolism lack single-cell resolution, obscuring metabolic heterogeneity and its connection to immune phenotype and function. To that end, we utilized high-dimensional, antibody-based technologies to simultaneously quantify the single-cell metabolic regulome in combination with phenotypic identity. Mass cytometry (CyTOF)-based application of this approach to early human T cell activation enabled the comprehensive reconstruction of the coordinated metabolic remodeling of naive $CD8^+$ T cells and aligned with conventional bulk assays for glycolysis and oxidative phosphorylation. Extending this analysis to a variety of tissue-resident immune cells revealed tissue-restricted metabolic states of human cytotoxic T cells, including metabolically repressed subsets that expressed CD39 and PD1 and that were enriched in colorectal carcinoma versus healthy adjacent tissue. Finally, combining this approach with multiplexed ion beam imaging by time-of-flight (MIBI-TOF) demonstrated the existence of spatially enriched metabolic neighborhoods, independent of cell identity and additionally revealed exclusion of metabolically repressed cytotoxic T cell states from the tumor-immune boundary in human colorectal carcinoma. Overall, we provide an approach that permits the robust approximation of metabolic states in individual cells along with multimodal analysis of cell identity and functional characteristics that can be applied to human clinical samples to study cellular metabolism how it may be perturbed to affect immunological outcomes.

TH63. Human immune system heterogeneity across the lifespan at the single cell resolution

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Immune cell molecular changes associated with maturation and aging are not well characterized. Using single-cell RNA-seq, we profiled ~600k PBMC collected from 81 healthy individuals from two months to 90 years of age. We examined how transcriptomic patterns within different immune cell-types vary with age. Infants (< 18 months of age) PBMCs showed distinct transcriptomic patterns, defined by expansion of naïve T and B cells, appearance of a discrete transitional CD24+ B cell subset, and contraction of myeloid compartments (e.g. monocytes and DCs), memory B and T cells and cytotoxic CD8+ T cells. PBMCs from older adults (65-90 years of age) exhibited an accumulation of highly differentiated TEMRA CD8+ T cells. Further characterization of purified CD8+ T cells from an independent set of older adults, revealed that TEMRA CD8+ T cells concomitantly express NK receptors, cytotoxic-related genes and senescence associated molecules, challenging the widely-held view that senescent-like CD8+ T cells are dysfunctional. Taken together, our data provide new insights into human immune system heterogeneity across the lifespan.

TH74. Web-based data analytics tools for the high-dimensional study of human Immunome development

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Mass cytometry (CyTOF) is a powerful immunomics technology that can characterise immune cell repertoires of multiple samples in a single experiment. Inspired by the idea that a better understanding of immune cell subsets during healthy human development can provide new insights into disease mechanisms, we have applied CyTOF to quantify 63 protein expression in nearly 200 peripheral blood samples of healthy donors from birth to old age. To build and data-mine the EPIC (Extended Poly-dimensional Immunome characterisation) immune atlas (Nature Biotech accepted), we developed a computational pipeline using the R Shiny platform, which provides intuitive GUIs, can be run on desktops and web servers, and relieves users of the need to deal with source codes. Data structures called immune maps are used to integrate single cell protein expression data of multiple samples with clinical metadata and phenotypic information inferred from automated clustering and assisted cell type annotation. Clustering is combined with batch effect correction to reduce technical while preserving biological variations. Subsequent single cell exploratory data analysis and statistical tests help to

identify cell populations whose frequencies vary significantly between different age groups. We characterised changing frequencies of immune cell populations from newborns to elderly using two different antibody panels. Data mining revealed a series of developmental patterns, such as increases at the start and decreases at the end of childhood of double negative (CD4- CD8-) T cells (DNTs) and NK cells, suggesting that these cells, apart from host defense, may also play a role in tissue growth.

TH375. High-throughput RNA sequencing reveals major alterations of inflammatory molecules involved in pathogenesis of psoriasis

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Psoriasis is complex skin disease involved with genetic predisposition, a variety of environmental triggers, and inflammation. Inflammation process of psoriasis is associated with abnormal communication between infiltrating immune cells and activated keratinocytes. Cytokine and chemokine networks are associated with cellular communication and controlling inflammatory pathways in psoriasis. Recently, high-throughput complementary DNA sequencing (RNA-seq) has introduced with high that accuracy and precision. However, data are still limited and additional researches associated psoriasis treatments are still needed. We would like to explore the transcriptomic of landscape of psoriasis. Tissue samples from patients with psoriasis will be collected before and after treatment with narrowband (NB) UVB.

MAPKAPK3, OASL, SERPINB4 were significantly upregulated in mature lesion of psoriasis after successfully treated with NB-UVB. Whereas PRKDC, ATF2, GSTA3 were downregulated. STAM, FNDC3A, LAMTOR4, FNDC3A were significantly decreased after successfully treated with NB-UVB at perilesional lesion. PKM and AP1S2 were upregulated at the perilesional lesion.

Combining microarray data on biopsies from psoriasis patients with pathway analysis allowed us to the identification of mechanisms that may be important in psoriasis skin. Furthermore, these results may reflect primary effect of NB-UVB treatment of psoriasis.