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ABSTRACT SUPPLEMENT

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Allergy

Th100. Cashew and Shrimp Oral Immunotherapy-induced Changes in Allergen-reactive CD4+ T Cells

Sheena Gupta[1], Sharon Chinthrajah[1], Andrea Fernandez[1], Abhinav Kaushik[2], Holden Maecker[1], Monali Manohar[1], Sofia Maysel-Auslander[1], Kari Nadeau[2] and Sayantani Sindher[1]

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Abstract Text: Among food-allergic events, nuts like cashew and shellfish such as shrimp, are often implicated in anaphylaxis. Here we look at immunological responses with oral immunotherapy (OIT) treatment, specifically changes in allergen-specific T cells. We studied 52 participants with allergies to either cashew (40) or shrimp (12) from an open-label, Phase 2 clinical trial- MOTIF(NCT03504774). 52 weeks of OIT was followed by 6 weeks of avoidance and oral food challenges were performed at baseline, post-OIT (Week 52) and post-avoidance (Week 58). Participants were assessed for their ability to achieve allergen desensitization and sustained unresponsiveness. Multicolor flow cytometry identified allergen-reactive ($CD69^+CD40L^+$) $CD4^+$ T cells post specific allergen stimulation at all three timepoints. At week 52 and week 58, there was a significant decrease in cashew-reactive pathogenic Th2 $CD4^+CRTH2^+$ cells from baseline ($p = 0.0485$). We also used cashew- and shrimp-epitope specific tetramers to immunophenotype specific $CD4^+$ T cells among unstimulated PBMCs from compatible participants to 5 HLA subtypes. The majority (> 85%) of $tetramer^+$ $CD4^+$ T cells exhibited memory (CD45RA-) phenotype with Th2-A polarization ($CRTH2^+$ CXCR3- CXCR5-) at baseline. There was a significant decrease ($p=0.0474$) in frequency of $tetramer^+$ $CD4^+$ T cells with a baseline frequency of 0.77%, reducing to 0.41% and 0.22%, at weeks 52 and 58 respectively. We also observed a significant increase in the frequency of $CCR6^+$ $tetramer^+$ $CD4^+$ T cells ($p=0.0387$) at weeks 52 and 58. These results could potentially enhance the prospect for better OIT for other allergens by providing insight into the immune mechanisms behind it.

Th101. Discovery and Characterization of a Novel Engineered Fc-fused IgE Cleaving Enzyme for Treatment of IgE Mediated Diseases

Nam Le, Purvi Mandi, Jung-Eun Shin, Jordan Anderson, Minasri Borah, Soumya Bengeri, Andita Newton, Grace Carey, Allison Colthart, Nathan Rollins, Ryan Peckner, Jyothsna Viswesaraiah, Julia Manasson, John Sundy, Nathan Higginson-Scott, Kevin Otipoby, Yi Xing, and Ivan Mascanfroni

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Abstract Text: The dysregulation of humoral immune mechanisms results in pathogenic IgE production that contributes to a range of allergic and atopic diseases such as food allergies, acute anaphylaxis, allergic asthma, allergic rhinitis and chronic spontaneous urticaria. We present a novel Fc-fused bacterially-derived IgE protease that was engineered using a proprietary machine learning enabled platform to reduce immunogenicity and improve manufacturability while maintaining potency. The protease selectively cleaves IgE, eliminating it from circulation, the cell-surface and immune-complexes, and provides a novel therapeutic opportunity to treat IgE-mediated inflammation. The engineered Fc-fused IgE protease was identified using our proprietary IMPACT platform and was characterized *in vitro* to determine its ability to cleave IgE using MSD and flow cytometry-based cleavage assays. Immunogenicity was determined using T cell proliferation assays. Pharmacokinetics, pharmacodynamics and *in vivo* efficacy were tested using relevant preclinical models. The engineered Fc-protease selectively cleaves IgE while maintaining stability and demonstrating low immunogenicity. It cleaves soluble IgE in human plasma with high potency, IgE^+ BCR and IgE bound to CD23/FcERII in B cell lines. Notably, this protease shows extended pharmacokinetics and efficacy in preclinical models of local and systemic acute anaphylaxis, suggesting an impact on IgE-mediated effector functions. Given its ability to simultaneously address multiple aspects of IgE pathogenesis and its efficacy in preclinical models of anaphylaxis, the engineered Fc-protease offers a new approach to targeted therapy for allergic and atopic diseases where IgE is a key driver.

Th102. Friend or Foe? Tissue Resident Memory CD8$^+$ T Cells with Patient Specific TCRs Induce Allopurinol Associated Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

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Abstract Text: Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN) is a rare, T cell mediated, HLA class I restricted, drug-induced hypersensitivity reaction. SJS/TEN is characterized by keratinocyte death resulting in blistering and sloughing of skin and other mucous membranes. There is currently no established treatment for SJS/TEN other than cessation of causal drug and implementation of supportive care. The gout management medication, allopurinol (ALP), is one of the most common causes of SJS/TEN globally. HLA-B*58:01 is strongly associated with the development of ALP-SJS/TEN; however, the specific mechanisms involved in disease pathogenesis are ill-defined and carriage of the allele is not a complete risk factor. We have therefore established a 10x single-cell-TCR-RNA-CITE-seq pipeline paired with functional approaches in patients with confirmed HLA-B*58:01 restricted ALP-SJS/TEN to identify additional risk factors associated with disease pathogenesis. Single-cell analysis reveals ALP-SJS/TEN blister fluid and affected skin to be heavily infiltrated by CD69$^+$ CD103$^+$ CD8$^+$ TRM T cells expressing patient specific TCRs as well as a mixed population of pro-inflammatory and anti-inflammatory macrophages. Moreover, spatial transcriptomics indicate these cytotoxic CD8$^+$ TRM T cells to be localized to the dermal-epidermal junction in affected skin, suggesting the involvement of cross-reactive viral epitopes in disease pathogenesis. *In vitro* modeling reveals that some, but not all, patient specific TCRs are activated by drug in the presence of HLA-B*58:01. Together, these findings suggest that other factors, such as specific peptides at sites of tissue damage, may be involved in disease pathogenesis. Further studies could indicate additional risk factors associated with ALP-SJS/TEN and facilitate targeted therapeutic discovery.

Th103. TCF1 Expression Defines Functional Th2 Heterogeneity in Tissues with Type 2 Inflammation

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Abstract Text: Repetitive exposure to antigen in chronic infection and cancer drives T cell exhaustion, limiting adaptive immunity. In contrast, ongoing antigen exposure in allergic disease results in aberrant, sustained T cell responses. We recently defined a novel human Th2 cell state, the Th2 multipotent progenitor, through single cell RNA sequencing (scRNAseq), single cell TCR sequencing, and *ex vivo* function studies. These cells co-express TCF1 and LEF1, transcription factors critical for chronic and memory T cell responses, and differentiate into effector cells while maintaining self-renewal capacity. We propose that this chronically activated tissue memory population may sustain chronic inflammation in type 2 disease. To understand the factors that modulate Th2 progenitors, we established a mouse model of chronic pulmonary type 2 inflammation. We found that type 2 inflammation was broadly sustained over months despite chronic stimulation. Th2 cells in the lung parenchyma during chronic inflammation were heterogeneous and included a TCF1($^+$)ST2($^-$) compartment reminiscent of the human Th2-MPP population. These cells could self-renew for weeks without contribution from the draining lymph node. scRNAseq analysis of acute and chronic responses defined divergent effector programs, as well as the transcriptional signature of tissue Th2 progenitors. After adoptive transfer, parenchymal Th2 progenitors could self-renew and differentiate into TCF1($^+$)ST2($^+$) and TCF1($^-$)ST2($^+$) effector cells. Moreover, Th2 progenitors, but not Th2 effectors or ILC2s, were sufficient to initiate and sustain allergic inflammation over time. Our findings define the function of progenitor Th2 cells with the capacity to sustain chronic type 2 inflammation in the face of ongoing antigen exposure

Th104. The human colon as the source of IgE and the role of microbiota in food-sensitized patients

Guillermo Docena[1], EUGENIA ALTAMIRANO[2], VIBIANA BERNEDO[2], MARCELA GARCIA[2], LUCIANA GUZMAN[2], CECILIA MUGLIA[1], JULIAN VACCARO[1] and Guillermo Docena[1]

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Abstract Text: Food allergies are provoked by an aberrant immunological reaction to food components. Evidences of IgE production in the human gut and IgE memory cells are scarce. We found that colorectal polyps from children with rectal bleeding and sensitized to food allergens are dominated by an inflammatory cell infiltration. We aimed to study immunoregulatory mechanisms and IgE synthesis in polyps. Polyps were removed and adjacent biopsies were grabbed. We found that 44% of patients had pedunculated colorectal polyps, 83% showed the presence of IgE, and the stroma had a dense infiltration of eosinophils and mononuclear cells. Our findings showed a significantly higher frequency of IgE^+CD138^+ cells (B cells) and IgE^+CD138^- (eosinophils) within polyps than in the adjacent tissue. Germinal centers with proliferation, hypermutation and class-switch of B cells, along with markers for IgE switching, and IgE^+ memory B cells ($CD45^+CD19^+CD138^+CD27^+IgD^-IgE^+$ cells) were detected in 85.7% of polyps analyzed. The polyp stroma showed a type-2 inflammatory environment, with high levels of IL-4 IL-5, IL-33 and TSLP and a high IL-13/IFN-γ ratio compared to the surrounding tissue, and the presence of milk-specific IL-13-producing T cells expressing CCR9, concurrent with CCL25 polyp expression. Finally, the whole microbiota and that attached to the polyp tissue was similar to that described in food- allergic patients with a lower frequency of Firmicutes and enhanced Bacteroidetes. In conclusion, we found that IgE is produced in polyps with IgE^+ B cell memory, likely influenced by the strong type-2 inflammation and microbiota associated with food allergy.

Th105. The Role of CCL21 in Environmental Allergies and Infection-Associated Chronic Illnesses

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Abstract Text: Environmental allergies (EA), characterized by recurrent multi-system symptoms triggered by low-dose antigens, significantly impact both individual health and socioeconomic stability. Despite affecting up to 25.6% of the population, the underlying immune mechanisms contributing to EA remain poorly understood. The MIT MAESTRO clinical study investigates immune pathways associated with infection-associated chronic illnesses (IACI)—including chronic Lyme Disease and Long COVID—through cutting-edge techniques such as neurocognitive assessments, pathogen profiling, and genomic sequencing. Within this study, we utilized TruDiagnostic's analysis platform, which applies Illumina 850k methylation arrays to model protein and metabolite levels. This allowed us to examine methylation patterns and their relationship to EA severity, with the goal of identifying biomarkers and gaining insights into underlying immune mechanisms. EA severity is measured by a composite score across 28 environmental allergy-related questions, such as mold, heat, and fragrances. We observed a significant negative correlation (Spearman $\rho = -0.62$, $p < 0.05$), adjusted for age and sex, between CCL21 methylation levels and EA severity. CCL21 is a chemokine that plays a critical role in regulating the migration of immune cells from tissues into lymphatics. It is upregulated in the skin of subjects exposed to irritants, and mice deficient in CCL21 develop a wide range of allergic conditions, including asthma. Our findings suggest that altered methylation of CCL21 may contribute to the development and pathogenesis of EA and its intersection with IACI. Further studies focused on an EA-specific cohort are currently underway to clarify the immune mechanisms involving CCL21 and identify potential therapeutic targets.

Th131. Agnostic Sampling of Singaporean Cohort Reveals Loss of Tolerance in T/B Lymphocytic Axis in House Dust Mite Allergic Individuals

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Abstract Text: In Singapore, 70% of the population exhibits positive serological (IgE^+) response to House Dust Mites (HDM), why certain individuals who are immunologically sensitized to HDM, can elicit a non-allergic response by maintaining tolerance, remains unknown. This study aims to decipher the T/B lymphocytic immune subsets that contributes to the regulatory axis in HDM allergy with the aid of high parametric CyToF analysis on an agnostic Singaporean cohort. This population was categorised through clinical scoring of plasma IgE reactivity to Derp1 (n=16 IgE-$Derp1^+$, n=16 IgE-Derp1-) and the PBMCs was interrogated with a dedicated T/B cell CyToF panel of 43 markers. Global overview of main immune lineages reveal a distinct gross dysregulation in T and monocytic compartment. Focusing on CD4 Tregs, we discovered there was a distortion in Treg memory and naive ratio, with allergic individuals reflecting a drop in memory Tregs ($p < 0.01$). In-depth cluster analysis of the Treg compartment, revealed a distinct deficit in the memory $CCR4^+CCR6^+$CD154- Treg subset within allergic individuals ($p < 0.001$), displaying activation marker ($HLADR^+$) and suppressive phenotypes ($ICOS^+Tigit^+$). Importantly, they correlate negatively ($p < 0.0001$) with plasma IgE-$Derp1^+$ levels. Separately within the CD8 compartment, we discovered a similar regulatory deficit in germinal centre targeting $CD8^+CD45RO^+CD27^+CXCR5^+$ (TfH) with TC1 regulatory capacity ($TNFa^+$, $IFNg^+$) in allergic individuals. Overall, we present data on regulatory immune subsets reflecting the loss of tolerance in T/B lymphocytic axis impacting HDM allergic individuals, that could possess therapeutic potential.

Tu100. The Role of the CH25H/EBI2 Axis in the Recruitment of Mast Cell Progenitors During Type 2 Inflammation

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Abstract Text: Basophils, eosinophils, and mast cells (MCs) are granulocytes which expand in the airways during Type 2 Inflammation (T2I) -associated diseases, where they are thought to play a major role in disease pathobiology. While the chemotactic signals responsible for recruitment of basophils and eosinophils to the inflamed tissue are well characterized, the signals directing MC progenitor (MCp) recruitment remained unknown. Single-cell RNA sequencing of human peripheral MCps reviled high expression levels of Gpr183, encoding the receptor EBI2. Venous endothelial cells in human sinus tissue and asthmatic airways exhibit high expression of cholesterol 25-hydroxylase (CH25H), an enzyme necessary for production of EBI2 ligands. Mouse MCps and mouse endothelial cells exhibit similar expression pattern of Gpr183 and Ch25h. To further assess the role of the Ch25h/EBI2 axis in pulmonary MC expansion, we adapted an approach, in which mice were IV-injected with FITC-labelled anti-CD45 to distinguish immune cells within the vasculature, versus immune cells within the lung parenchyma to track MCp recruitment upon aeroallergen challenge. Following dust mite-induced allergic lung inflammation, we find that MCps in WT mice are quickly recruited to the lung parenchyma and retained, where they undergo phenotypic maturation. In contrast, MCp recruitment to the lung parenchyma was reduced by 97% in mice with a targeted deletion of EBI2 in hematopoietic cells (Vav1Cre; EBI2FL/FL) and by 95% in ch25h deficient mice. EBI2/Ch25h deficiency had little to no effect on basophil or eosinophil recruitment, suggesting a selective role for the EBI2/Ch25h axis, in directing MCp recruitment from the vasculature during T2I.

Tu101. Type 2 Immune Response and Lyme Disease: Can Allergic-type Responses to Bacteria Trigger Infection-induced Hypermobility?

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Abstract Text: There are currently an estimated 476,000 cases of Lyme disease annually in the US, caused by the bacteria Borrelia burgdorferi (Bb). It is estimated that approximately 10% of people who have had Lyme disease go on to develop an infection-associated chronic illness, the underlying causes and long-term impacts of which remain poorly understood. In our Lyme disease mouse model, we discovered that C3H/HeJ mice mount significantly higher levels of anti-Bb IgE compared to C57BL/6 mice. As IgE is heavily involved in the Type II immune response, we further investigated the IgE epitopes and identified highly conserved epitopes that are conserved across spirochetes. Histological analysis of infected C3H mice also revealed IgE-dependent mast cell degranulation. Furthermore, we found that these mice develop atopic dermatitis (AD) on their tails, in sites with high levels of Bb load. Strikingly, we find that intradermal infection with Bb led to the development of AD followed by a gradual development of hypermobility in the tail, revealing infection-induced hypermobility which was not seen in uninfected age-matched C3H or age-matched B6 regardless of infection status. Moreover, we observed Bb in fresh mouse tissue under simultaneous label-free autoflourescent-multiharmonic microscopy in the connective tissues with extensive mast cell recruitment C3H mice suggesting that IgE-dependent mast cell-mediated connective tissue breakdown can result in infection-induced hypermobility.

Tu102. Atypical Presentation of Hereditary Alpha Tryptasemia: The Role of a Concurrent Auto-inflammatory Gene Variant

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Abstract Text: Background Hereditary Alpha Tryptasemia (HaT) is a genetic condition that arises from increased alpha-tryptase gene copy numbers (TPSAB1). Common clinical manifestations include mast cell activation symptoms such as anaphylaxis, flushing, and urticaria, as well as less frequently, more subtle or atypical features such as arthralgias, gastrointestinal symptoms, and low-grade fevers. Variants of unknown significance (VUS) in autoinflammatory genes (NLRP3, NOD2) may contribute to the presentation variability. Case Presentation A 32-year-old Caucasian French Canadian male presented with a twelve-year history of recurrent fevers, intermittent diarrhea, arthralgias, and fatigue. He had persistently elevated baseline tryptase (~20 ng/mL) and exhibited classic mast cell-mediated reactions, including a rash. Extensive workup ruled-out systemic mastocytosis (negative c-KIT mutation, bone marrow biopsy), autoimmune conditions, and infections. Genetic testing confirmed HaT (duplication of alpha-tryptase coding exons in TPSAB1) and identified a VUS in two autoinflammatory genes (NLRP3, NOD2). Complete response was observed following treatment with second-generation H1-antihistamines and histamine H2 blockers. Over two years of follow-up, his arthralgias, fevers, and gastrointestinal complaints remained fluctuating but controlled, with no severe reactions. Discussion This case highlights how HaT can manifest predominantly with constitutional and autoinflammatory-like symptoms rather than the classic allergic or anaphylactic phenotype. The concurrent VUS emphasizes the potential genetic interplay that may influence symptom severity and expression. Conclusion Increased awareness of HaT's broad spectrum of presentations is crucial for timely diagnosis and management. Exploring genetic modifiers, including autoinflammatory variants, may shed light on individualized therapeutic strategies and improve outcomes for patients with elevated tryptase and atypical clinical features.

Tu103. Cysteinyl Leukotrienes Promote Anaphylaxis to Ingested Allergens

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Abstract Text: Food allergy affects approximately 8% of US children with increasing prevalence and limited therapeutic options. Although food-specific IgE is necessary for anaphylaxis in humans, it is not sufficient, indicating that other factors contribute to anaphylaxis susceptibility. We found that despite equivalent levels of food-specific IgE and anaphylactic response upon systemic challenge in both C57BL/6 and C3H/HeJ mouse strains, the C57BL/6 mouse strain is uniquely resistant to anaphylaxis following the ingestion of food allergens. By crossing C57BL/6 and C3H/HeJ mice and performing a genetic screen, we found that oral anaphylaxis resistance corresponded with allelic variants of Dipeptidase 1 (Dpep1). Dpep1 encodes the enzyme DPEP1, which catabolizes leukotriene D4 (LTD4). DPEP1 activity was significantly higher in intestinal tissue from C57BL/6 mice compared to C3H/HeJ mice despite equivalent protein levels. In addition, C57BL/6 mice had lower intestinal levels of LTD4. Genetic deletion of Dpep1 or treatment with exogenous LTD4 rendered C57BL/6 mice susceptible to oral anaphylaxis while blockade of leukotriene synthesis protected C3H/HeJ mice from to oral anaphylaxis. These data suggest that leukotrienes regulate susceptibility to oral anaphylaxis in mice. Ongoing research will determine whether altering the leukotriene pathway in humans can similarly prevent anaphylactic response to ingested allergens.

Tu104. Dysregulations in L-phenylalanine Metabolism Facilitate Pro-inflammatory th2 Phenotype in Severe Allergy

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Abstract Text: Changes in T cell metabolism guide and determine their fate. However, metabolism and metabolic reprogramming of CD4$^+$T cells in allergic diseases remains poorly understood. Hence, we performed metabolomics of circulating memory CD4$^+$Teff and Treg cells from healthy subjects which revealed significant enrichment in amino-acid and L-phenylalanine (Phe) metabolism related pathways. Consequently, we studied effect of Phe on CD4$^+$T cell energy metabolism and observed that Phe significantly enhanced total and memory CD4$^+$T cell glycolysis while limiting OXPHOS. Phe also limited memory CD4$^+$T cell proliferation via an Interleukin-4-induced-1 oxidase (IL4I1)-dependent mechanism which was confirmed using siRNA experiments. Next, we studied Phe induced changes in Th2 and Treg cell energy metabolism, using recently developed Single Cell ENergetIc metabolism by profilIng Translation inhibition (SCENITH), and observed a significant increase in Th2 cell glycolytic capacity coupled with a decrease in mitochondrial dependence. In *in vitro* differentiated Th2 cells, Phe limited their proliferation and transcription of critical type 2 factors such as mTOR, IL-4, IL-5, and IL-13. Additionally, Phe inhibited phosphorylation of mTOR and expression of CD161, a documented pathogenic Th2a marker. Metabolomics and *ex vivo* assessment of CD4$^+$Teff cells and serum of allergic patients revealed significantly lower intracellular abundance of Phe in circulating memory CD4$^+$Teff cells of a subset of severe allergic patients, elevated serum Phe concentration, and a significant negative correlation between LAT1, an important Phe transporter, and serum Phe. Altogether, these data

highlight that Phe regulates Th2 cell metabolism and development of pathogenic Th2 cells and this mechanism is impaired in severe allergy.

Tu105. Early-life Dysbiosis and Its Impact on Neuroimmune Interactions in Allergic Asthma

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Abstract Text: Early-life dysbiosis is linked to increased susceptibility to allergic asthma. In murine models, perinatal exposure to vancomycin (an antibiotic depleting short-chain fatty acid-producing bacteria) exacerbates allergic responses in adulthood. Dysbiosis induced in adulthood does not affect immunological skewing, highlighting a critical developmental window for the influence of microbiota on immune and tissue maturation. Many immune cells that reside near nerves express receptors for neuromediators. While dysbiosis impacts the enteric nervous system, its effects on autonomic function in distant tissues remains unclear. This study examines neuroimmune interactions in the context of early-life dysbiosis, focusing on neuroimmune mediators driving Th2 responses. Methodology: 1. Dysbiosis Model: C57BL/6J breeding pairs receive vancomycin (200 mg/L) in drinking water; pups are reared on treatment water until weaning. 2. We optimized a spectral flow cytometry panel to comprehensively profile immune cells in lung tissue from male and female mice. 3. Bulk proteomics is used to identify neuroimmune mediators in dysbiotic lungs; key mediators (serotonin, Substance P, CGRP) are validated via qPCR. 4. Immunohistochemistry will illustrate spatial relationships between IgE-bearing immune cells and autonomic nerve fibers in dysbiotic versus normobiotic lungs. Preliminary flow cytometry results demonstrate increased FcεRIα-bound IgE expression on immune cells in female dysbiotic lungs, with lesser effects seen in males. As FcεRIα also appears on lung-innervating vagal afferents (Crosson T, et al. JACI. 2021), this suggests a neuroimmune axis mediating enhanced Th2 responses. This research will reveal the impact of dysbiosis on neuroimmune interactions, potentially guiding the use of neuromodulatory drugs for allergic disease management.

Tu106. Eosinophilic Colitis in a Patient with Asthma: A Rare Case of Gastrointestinal Eosinophilia

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Abstract Text: Eosinophilic gastrointestinal diseases (EGIDs) are rare inflammatory conditions marked by eosinophil-predominant inflammation in the gastrointestinal tract. Eosinophilic colitis, a type of EGID, often presents with nonspecific symptoms such as abdominal pain, diarrhea, and weight loss. Due to the rarity of EGIDs, early recognition and treatment is particularly challenging. A 37-year-old woman with asthma presented with intermittent abdominal pain and non-bloody diarrhea. She was recently hospitalized for a severe asthma exacerbation that required intubation, though she made a full recovery. Laboratory studies revealed marked eosinophilia (AEC 3.6 $x10^9$/L, 82%), elevated IgE (1759), and mild CRP elevation (1.01). Imaging showed small bowel enteritis and pancolitis. Stool studies and infectious workup were negative. Targeted biopsies revealed pronounced eosinophilia in the colon, including focal abscess formation and crypt epithelial infiltration. The patient received IV solumedrol with rapid symptom resolution, normalization of eosinophilia, and symptomatic improvement. She was transitioned to oral prednisone with continued symptom control. This case highlights eosinophilic colitis as a rare, but significant consideration in patients with atopic diseases presenting with unexplained GI symptoms. IV corticosteroids followed by an oral taper led to significant clinical improvement, though the optimal steroid dose and taper duration remain uncertain given the infrequency of eosinophilic colitis. Given the patient's history of asthma, biologics may provide alternative treatment options for long-term management rather than steroids. Identifying unusual presentations of EGIDs allows for early diagnosis and effective treatment as well as adding to the limited literature on this disease.

W106. Evaluating Allergen-mediated MCT and MCTC Activation During Immunotherapy

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Abstract Text: Mast cells (MCs) can be categorized into two distinct populations: subepithelial MCs that co-express tryptase and chymase (MCTC) and epithelial MCs that express tryptase alone (MCT). MCT are the principal phenotype found in airway epithelium, suggesting they are central drivers of aeroallergy. However, all current *in vitro* systems give rise to MCTC. MC subsets are transcriptionally distinct from each other and differentially express key genes associated with IgG-driven inhibition, suggesting differential sensitivity to allergen-mediated desensitization. To test this, we selectively differentiated MCT and MCTC from peripheral blood-derived $CD34^+$ cells, sensitized them using patient serum samples, and evaluated: 1) if differences in activation exist between MC subsets, and 2) whether immunotherapy improves MC subset activation. For this study, we utilized serum samples from a recent study of cat allergic individuals evaluating effects of subcutaneous immunotherapy (SCIT) on patient responses to cat dander. Our initial studies indicate that *in vitro* differentiated MCT exhibit a 2-fold increase in degranulation compared with MCTC following activation with cat dander antigen. This enhanced MCT activation is especially pronounced when sensitized with serum from patients with low cat-specific IgE titers. Sensitizing MC subsets with SCIT treated patient serum samples improved MCT activation significantly more than MCTC activation, suggesting that MC subsets may exhibit differential sensitivity to activation during desensitization.

W107. Evaluating Variability in Protein Content of At-home Peanut Allergy OIT Doses

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Abstract Text: Background: Peanut oral immunotherapy (OIT) uses controlled doses of peanut protein to build tolerance. At-home doses are prepared by mixing peanut powder (PB2) into applesauce. Measuring spoons are only available down to 1/64 tsp, but smaller doses are obtained by diluting 1/64 tsp PB2 (~16 mg protein) in applesauce. Currently, doses of ~4 mg protein (1/256 tsp; 1:4 dilution) are used for OIT. Our study aimed to evaluate variability in protein content across dilutions and assess whether smaller doses are feasible using this method. Methods: PB2-applesauce dilutions were prepared at 1:1, 1:2, 1:4, or 1:8 (~16, ~8, ~4, or ~2 mg protein respectively). Protein content was analyzed via SDS-PAGE electrophoresis and quantified using ImageJ. Results: Variability was assessed by calculating the coefficient of variance for total protein and the major peanut allergen, Ara h2. For total protein, the CVs were 10.22% (1:1), 13.28% (1:2), 34.86% (1:4), and 51.44% (1:8). When analyzing the Ara h2 protein band alone, variability was 16.38% (1:1), 26.52% (1:2), 15.90% (1:4), and 29.28% (1:8). Conclusion: These findings suggest that current at-home OIT doses may require further evaluation for consistent protein delivery. With dilutions using this technique, variance is inversely proportional to concentration, suggesting that patients sensitive to low doses could be at risk of reactions. However, lower variability of the major allergen, Ara h2, calls into question the clinical significance and may help to account for a low rate of adverse reactions observed to date.

W108. Glutamine Metabolism and mTOR Signaling Defects in CARD11-Mutant T Cells: Implications for Allergic Disease Treatment

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Abstract Text: Genome-wide association studies have identified members of the CARD11/MALT1/BCL10 (CBM) complex as susceptibility loci for allergic disorders. Loss of CBM complex function impairs lymphocyte receptor–mediated activation of NFκB as well as glutamine uptake—thereby selectively reducing signaling in mTORC1, but not mTORC2. Because patients with NFkB deficiency do not develop significant Th2 phenotypes, we therefore investigated how mTORC1/mTORC2 dysregulation might lead to Th2-associated allergic predisposition. Using click chemistry with bio-orthogonal glutamine analogues, we observed reduced glutamine uptake upon T cell receptor (TCR) stimulation in T cells from CARD11 patients, which was associated with reduced mTORC1 signaling, and preserved to elevated mTORC2 activation. Cultures of TCR-stimulated naive CARD11DN CD4 T cells lead to increased GATA3 expression compared to controls, which was inhibited via hyperphysiologic exogenous glutamine. Bulk RNA-seq of these cultures showed significant differences in expression of genes involved in PI3 kinase/AKT and autophagy pathways. Furthermore, 24 hours of glutamine deprivation in proliferating healthy control $CD4^+$ T cells led to reduced mTORC1 signaling, NRF2-p62 pathway activation, increased Th2 cytokine production and mTORC2 signaling. Similarly, CARD11 mutant mice exhibited elevated nuclear NRF2 protein levels and p62 transcription compared to wild-type controls. Notably, NRF2 inhibitors suppressed the elevated Th2 cytokines in these mutant mice, mirroring our findings in human samples. Decreased CBM function may promote Th2 phenotypes by impairing glutamine-mediated mTORC1 activation, while simultaneously enhancing mTORC2 and NRF2 activation. These results highlight a potentially fundamental immunometabolic pathway in Th2-mediated disease. Strategies to enhance intracellular glutamine or inhibit NRF2 and mTORC2 may prove therapeutic in such settings.

W109. IL-2 Mutein Prevents the Development of Allergic Asthma

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Abstract Text: Allergic asthma is a chronic inflammatory disease characterized by an aberrant type 2 immune response to aeroallergens. Type 2 cell-mediated responses drive allergic asthma due to an imbalance of allergen-specific effector and regulatory cell responses. Regulatory T cells (Tregs) are potent suppressors of inflammation and are attractive targets to treat allergic asthma. Interleukin-2 (IL-2) is essential for Treg function and IL-2 muteins, such as Fc.mut24, have been engineered to increase Treg selectivity and half-life. We hypothesized that targeting Tregs with IL-2-Fc.mut24 could tip the balance and lead to suppression of allergen-induced type 2 airway inflammation. To assess this hypothesis, Fc.mut24 was given s.c. to C57BL/6 mice 3 days prior to sensitization with intranasal (i.n.) house dust mite (HDM) followed by i.n. HDM challenges on days 7–11 and harvested on day 15. Fc.mut24 treatment prevented the development of allergen-specific Th2 cells in the draining mediastinal lymph node and lung and prevented allergic airway inflammation and airway hyperreactivity. Fc.mut24 treatment prior to HDM sensitization had a long-term effect, as treated mice did not develop a Th2 cell recall response and lung inflammation following allergen rechallenge on day 42. In contrast, mice that first received Fc.mut24 in the memory phase - after HDM sensitization and challenge - did not suppress the Th2 cell recall response and developed airway inflammation following allergen rechallenge. In conclusion, Fc.mut24 prevented the development of allergic airway inflammation, but once allergen-specific Th2 cell responses are established, Fc.mut24 treatment was unable to suppress allergen-induced allergic airway inflammation.

W110. Microbial Intestinal Dysbiosis Drives Long-term Allergic Susceptibility by Sculpting an ILC2-B1 Cell-Innate IgE Axis

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Abstract Text: Background: The abundance and diversity of intestinal commensal bacteria influence systemic immunity with impact on disease susceptibility and severity. For example, loss of short chain fatty acid (SCFA)-fermenting bacteria in early life (humans and mice) is associated with enhanced type 2 immune responses in peripheral tissues including the lung. Objective: Our goal was to reveal the microbiome-dependent cellular and molecular mechanisms driving enhanced susceptibility to type 2 allergic lung disease. Methods: We used low-dose vancomycin to selectively deplete SCFA-fermenting bacteria in wild-type mice. We then examined the frequency and activation status of innate and adaptive immune cell lineages with and without SCFA supplementation. Finally, we used ILC2-deficient and signal transducer and activator of transcription 6 (STAT6)-deficient transgenic mouse strains to delineate the cellular and cytokine pathways leading to enhanced allergic disease susceptibility. Results: Mice with vancomycin-induced dysbiosis exhibited a 2-fold increase in lung ILC2 primed to produce elevated levels of IL-2, -5, and -13. In addition, upon IL-33 inhalation, mouse lung ILC2 displayed a novel ability to produce high levels of IL-4. These expanded and primed ILC2s drove B1 cell expansion and IL-4-dependent production of IgE that in turn led to exacerbated allergic inflammation. Importantly, these enhanced lung inflammatory phenotypes in mice with vancomycin-induced dysbiosis were reversed by administration of dietary SCFA (specifically butyrate). Conclusion: SCFAs regulate an ILC2-B1 cell-IgE axis. Early-life administration of vancomycin, an antibiotic known to deplete SCFA-fermenting gut bacteria, primes and amplifies this axis and leads to lifelong enhanced susceptibility to type 2 allergic lung disease.

W111. Novel Bile Acid Suppresses Neutrophilic Airway Inflammation in Non-type 2 Asthma

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Abstract Text: Asthma affects more than 300 million individuals worldwide. Broadly, asthma can be characterized by eosinophilic T2 and neutrophilic non-T2 endotypes. Patients with non-T2 asthma show severe symptoms, lung neutrophilia, and poor responsiveness to the current gold-standard treatment for asthma including corticosteroids. The key pathogenic cells are group 3 innate lymphoid cells (ILC3) and T helper 17 (Th17) cells that express the transcription factor RORγt and play crucial roles in mediating neutrophilic inflammation. Isolithocholic acid (isoLCA) - a microbiome-derived secondary bile acid - is a reversible inhibitor of RORγt and shows efficacy in modulating Th17-driven intestinal inflammatory diseases. Here, we investigate the potential of isoLCA to inhibit RORγt in pathogenic ILC3 and Th17 cells in a murine model of non-type 2 asthma. High-dimensional spectral flow cytometry and immunohistochemistry reveal that oral administration of isoLCA significantly reduces airway neutrophilia. Mechanistically, isoLCA inhibits RORγt expression in the lungs, leading to reduced pathogenic ILC3 and Th17 cell populations, and lowers IL-17A levels in lung CD4 T cells and ILCs. Our results show that isoLCA attenuates neutrophilic airway inflammation in non-type 2 asthma by specifically inhibiting RORγt-driven pathways. These findings suggest that isoLCA has therapeutic potential as a novel agent suppressing RORγt to treat non-type 2 asthma, especially in cases unresponsive to corticosteroids.

W112. Pharmacological and Genetic Perturbation of Large Airway Epithelial Cells in Air-liquid-interface Culture

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Abstract Text: Airway epithelial barrier function is crucial for the development of Asthma and COPD pathogenesis. To identify mechanisms that drive epithelial dysfunction in Asthma/COPD, we aimed to establish a 3D *in vitro* culture system that recapitulates all airway epithelial cells types and enables functional studies of large airway epithelium. Protocols have emerged enabling airway-mimetic models by culturing primary lung basal cells in air-liquid interface

(ALI) culture. We established a platform to study the impact of pharmacological and genetic perturbation of airway epithelial cells in ALIs and to quantify the effect on cellular phenotype as well as cytokine/chemokine release. These ALIs formed a pseudostratified layer exhibiting key morphological characteristics of lung epithelium, including mucous secreting cells, basal cells and club cells. As previously reported, treatment with IL-13 induced goblet cell metaplasia, as evidenced by induction of $MUC5AC^+$ goblet cells. To allow for the interrogation of key pathways associated with epithelial function, we have optimized conditions to transduce basal cells with lentivirus without compromising ALI cellular diversity or ciliation. Using lentiviral vectors coexpressing Cas9 and CRISPR sgRNAs, we confirmed robust knockout of CD81, a control target gene in large airway basal epithelial cells and are currently testing the effects of gene knockout on IL-13, EGF, RAR agonism induced goblet and/or squamous metaplasia, key pathologies seen in COPD and asthmatic patients. This end-to-end platform of primary cell culture, organoid formation in ALIs, pharmacological and genetic perturbation, and multi-modal characterization forms a basis to identify and characterize new targets and mechanisms in Asthma and COPD.

Autoimmune Diseases

Th106. Engineered Cellular Therapies Targeting 9G4 Idiotope B Cells in Autoimmune Diseases and Lymphoma

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Abstract Text: Systemic lupus erythematosus (SLE) and many lymphomas are characterized by the expansion of 9G4 idiotope B cells. In SLE, these B cells are key sources of pathogenic autoantibodies. While CD19-CAR-T cell therapy has transformed the treatment of severe lupus, pan-B cell depletion increases the risk of infection. Precision immunotherapies targeting autoreactive 9G4 B cells, while preserving other B cells, could offer a safer alternative. Here, we developed synthetic immune receptor T cell therapies to selectively deplete 9G4 B cells in SLE and lymphoma. Ramos B cells were CRISPR-edited to replace their endogenous BCR with autoreactive BCRs from SLE patients. Anti-9G4 CAR or chimeric T cell receptors (cTCRs) were expressed in T cells from healthy donors or SLE patients using CRISPR/Cas12 (A). Co-culture of B cells with engineered T cells was performed to determine their potency and specificity. Interferon-γ and autoantibody levels were quantified using ELISA. Depletion of 9G4 B cells in SLE PBMCs was quantified by FluoroSpot and RepSeq. Both anti-9G4 CAR-T and cTCR-T cells efficiently eliminated autoreactive Ramos B cells (B-C) and their secreted autoantibodies (D-E), while sparing non-9G4 B cells. CAR-T cells showed significantly higher IFN-γ release and proliferation (F-G), but cTCR-T cells matched their cytotoxic potency with reduced cytokine release. Dose-dependent, selective depletion of 9G4 B cells in SLE PBMCs was confirmed by FluoroSpot (H) and repertoire sequencing (I-K). Anti-9G4 CAR-T and cTCR-T cells demonstrate potent, specific depletion of autoreactive and malignant 9G4 B cells. cTCR-T cells exhibit favorable safety characteristics, making them promising candidates for autoimmune diseases.

Th107. Induction of Antigen-specific T Cell Tolerance by CNP-104 Positively Affects Clinical Response in Primary Biliary Cholangitis (PBC)

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Abstract Text: Background: PBC is an archetypal autoimmune disease where the dominant autoantigen is mitochondrial PDC-E2. Hitherto, PBC treatment has been neither specific nor directed toward restoration of tolerance. CNP-104, a tolerogenic nanoparticle encapsulating PDC-E2, is hypothesized to restore tolerance to pathogenic T cells. Methods: We conducted a Phase 2a First-in-Human randomized controlled trial of CNP-104 in PBC patients unresponsive/intolerant to ursodeoxycholic acid. The 42 enrolled subjects were randomized 2:1 to receive two doses of CNP-104 or placebo (PBO) one week apart and were followed for 120 days to assess safety and treatment durability. Results: CNP-104 was safe and well-tolerated. Compared to PBO, the CNP-104 group had a reduced percentage of antigen-specific Th17 T cells and increased antigen-specific tolerogenic $CD8^+$ T cell counts (TIGIT, GARP). ALP, ALT, and total bilirubin were similar between groups. Clinically relevant effects on albumin levels, liver stiffness, and the rate of change in 5-year UK-PBC risk for liver outcomes were improved in the CNP-104 group compared to PBO. Conclusions: This trial is limited by small size and data variability. Induction of antigen-specific T cell tolerance, shown by reduced Th17 cells and increased TIGIT and GARP cells correlates with improved liver stiffness, albumin, and UK-PBC. These findings suggest the CNP-104 tolerogenic mechanism of action translates to clinical benefits. These data support future studies with this unique therapeutic platform to treat PBC.

Th108. Intraocular Differentiation of Effector B Cells in Uveitis

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Abstract Text: Uveitis encompasses a heterogeneous group of chronic inflammatory eye diseases. Although some patients benefit from B cell-targeted therapies, the precise role of B cells in uveitis remains elusive. To clarify their involvement, we conducted immune receptor profiling of B cells from the eyes and blood of uveitis patients and generated recombinant antibodies from selected ocular B cell receptors (BCRs) to investigate their antigen specificity. Our analysis revealed that $CD11c^{++}$ B cells and plasmablasts are significantly enriched and clonally

expanded in ocular samples compared to blood. These expanded ocular clonotypes demonstrated intraconal diversity, predominantly between CD11c^{++} B cells and plasmablasts, highlighting local expansion and differentiation within the eye. Notably, ocular B cells were often class-switched and their BCRs exhibited somatic hypermutation, indicating prior antigen exposure. CD11c^{++} B cells and plasmablasts shared heavy chain V segment skewing and somatic hypermutation patterns, suggesting parallel antigen-specific recruitment or expansion. Moreover, we identified convergent clonotypes across multiple patients, characterized by shared CDR3 sequence motifs, elevated somatic hypermutation levels, and reduced polyreactivity. This finding suggests that common antigens drive intraocular inflammation in uveitis patients.

Th109. Regulatory Role of JAK-1 Signaling in the Pathogenesis of Psoriatic Disease

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Abstract Text: In psoriasis/psoriatic arthritis (PsA) there is aberrant activation of specific T cell subpopulations (Th17/Th9/MAIT cells). Homing of T cells in skin/ joints induce plaque and pannus formation by secreting cytokines through JAK/STAT signaling pathway. New generation JAK-1 inhibitors such as upadacitinib/filgotinib have demonstrated clinical efficacy in psoriatic disease with less adverse effect. Role of JAK-1 in pathogenesis of psoriatic disease remains unaddressed. IL-2/IL-9/IL-22 are known to recruit JAK-1 and play critical role in pathogenesis of PsA. In cultured FLS (synovial cells) and KC (keratinocytes), rIL-22 induced increased phosphorylation of JAK-1 compared to the culture media ($p< 0.01$); further we observed (i) FLS proliferation and IL-6, IL-8 and MMP-3 production by FLS are induced by rIL-22 (ii) rIL-22 induced significant increase in proliferation of NHEKs compared to media only (< 0.005). KC/FLS pre-treated with upadacitinib had significantly lower proliferation than cells not pre-treated with upadacitinib at concentrations 1 μM and 5 μM (< 0.01). rIL-2 and rIL-9 upregulated the expression of phospho-Jak1 in the PBMC T cells by 2-fold ($p< 0.001$) as compared to the untreated control. rIL-2 and rIL-9 induced significant proliferation in PsA PBMCs compared to unstimulated PBMCs ($p< 0.001$). The rIL-2/rIL-9 induced proliferation of PsA PBMC T cells could be significantly inhibited by upadacitinib at 1μM concentration ($p< 0.01$). This study provides a novel insight about and independent role for JAK-1 signaling on (i) T cell dysregulation (ii) pannus and (iii) plaque formation in psoriatic disease and provides the mechanisms of actions JAK-1 inhibitor (upadacitinib) in the treatment of psoriasis and psoriatic arthritis.

Th110. Repertoire of Anti-nuclear Antigen (ANA) Reactive B Cells in Systemic Lupus Erythematosus

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Abstract Text: Objective. To gain insights into tolerance checkpoints, selection processes and developmental trajectories of autoreactive B cells, we studied the BCR sequences from thousands of anti-nuclear antigen binding (ANA)$^{+}$ and ANA- B cells from individuals with systemic lupus erythematosus (SLE). Methods. From a cohort of 13 patients with SLE, we sequenced B cell receptor (BCR) heavy chain variable regions and investigated the features of the IgH repertoire of ANA^{+} and ANA- B cells from naive, memory and age-associated B cells (ABCs), and from total plasmablasts. Results. The frequency of ANA^{+} B cells was similar in ABCs and naive B cells and higher in both than in memory B cells. ANA^{+} naive and ANA^{+} ABCs used different gene segments and have longer CDR3 sequences than ANA^{+} memory B cells and ANA- subsets. ANA^{+} ABCs and memory B cells have a lower frequency of somatic hypermutation (SHM), less strong selection and less activation induced deaminase (AID) targeting WRC hotspots compared with their ANA- counterparts. Patients with active disease have a lower frequency of SHM in

ANA+ ABCs and memory B cells and in ANA- ABCs. Conclusion. Our results suggest an immune checkpoint restricting the differentiation of ANA+ naive B cells into memory B cells and suggest that ANA+ ABCs originate from ANA+ naive B cells, potentially through an extrafollicular (EF) pathway. The EF pathway appears to be more prominent in patients with active SLE. Further, there is a dysregulation of somatic hypermutation in ANA+ antigen-experienced B cells in SLE.

Th111. S-4321, a Novel Dual-Cell Bidirectional PD-1:FcγRIIb Selective Agonist Antibody for the Treatment of Autoimmune Disease

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Abstract Text: The dysregulation of immune checkpoint receptors on T cells and antigen presenting cells (APCs) drives autoimmunity while receptor agonism is expected to restore immune homeostasis in diseases such as rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, and giant cell arteritis. PD-1 is a checkpoint receptor expressed on T cells, including Tfh and Treg subtypes. Strong PD-1 agonism requires super-clustering, which can be achieved by therapeutic antibodies binding to Fc gamma receptors (FcγR) on APCs. S-4321 is a novel dual-cell bidirectional (DcB) PD-1:FcγRIIb antibody that agonizes the inhibitory PD-1 receptor on T cells (Fab domain) and selectively engages the inhibitory FcγRIIb on APCs (Fc domain), eliciting an inhibitory signal at the immune cell synapse to re-establish homeostasis. S-4321 achieves prolonged agonism by binding to PD-1 with low affinity (similar to PD-L1) thus allowing for the retention of PD-1 expression on the T cell surface. This ensures continued presence of target and maintains a regulatory check on effector cell activity. In contrast, higher affinity PD-1 agonists decrease surface PD-1, associated with increased IL-2 production in SEB activated cultures. S-4321 also selectively engages FcγRIIb, avoiding the induction of proinflammatory cytokines and undesirable depletion of PD-1 expressing T cells, such as Tregs, by antibody-dependent cellular cytotoxicity (ADCC) through engagement of activating FcγR. In summary, S-4321 engages inhibitory receptors on both sides of the T cell-APC synapse, decreases T cell activation, preserves target expression and does not result in the elimination of PD1+ T cells important for the maintenance of a normal immune homeostasis.

Th112. SBT115301, a Second Generation CD2-targeting LFA3 Fusion Protein to Restore Immune Homeostasis in Autoimmune Disease

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Abstract Text: In autoimmune diseases, an imbalance of effector T cells (Teff) and regulatory T cells (Treg) contributes to inflammation and tissue destruction. CD2, an adhesion and co-stimulatory molecule, is highly expressed on Teff and at lower levels on Treg and naive T cells (Tn) and is an attractive target for depleting Teff at sites of inflammation. SBT115301 is a CD2-targeting drug consisting of the cognate receptor of CD2, Lymphocyte Function Associated Antigen-3 (LFA-3; CD58), fused with human immunoglobulin (Ig) G1-crystallizable fraction (Fc). SBT115301 induced antibody-dependent cellular cytotoxicity (ADCC) of CD2hi T cells and decreased the number of proliferating Teff responding to different stimuli *in vitro*. Nonclinical studies in non-human primates (NHP) demonstrated that SBT115301 decreased CD2hi-expressing effector memory (Tem) and central memory (Tcm)

cells, while Treg and Tn were less affected and there were no adverse events (AEs). In a first-in-human, randomized, single-dose escalation Phase 1 clinical trial studying both intramuscular (IM) and intravenous (IV) administration, the pharmacokinetics (PK) of SBT115301 was similar to other fusion proteins, and $CD4^+$ Tem and Tcm were preferentially depleted in participants who received the drug. Adverse events were minimal, aside from decreases of $CD4^+$ T cells in some participants in the highest dose IM and IV cohorts. In some individuals, immunogenicity, characterized by the formation of anti-drug antibodies (ADA), decreased exposure of SBT115301 without affecting its pharmacodynamics (PD). These data support further study of SBT115301 in autoimmune indications as a monotherapy or in combination with other drugs to restore balance to the immune system.

Th113. Serum C-type Lectin Domain Family 7 Member a Reflects Disease Activity in Patients with Systemic Lupus Erythematosus

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Abstract Text: [Aim] To investigate new biomarkers to monitor disease activity in systemic lupus erythematosus (SLE). [Methods] Inflammatory proteins were analyzed by proteome analysis in sera from 34 patients with SLE and 10 healthy controls (HC). Peripheral blood mononuclear cells were isolated, and proportions of 45 peripheral immune cell types were assessed by flow cytometry. Clinical data, including SLE disease activity index (SLEDAI), were collected, and correlations were evaluated. The selected protein level was measured in 21 patients with systemic sclerosis (SSc), as a disease control. [Results] From proteome analysis, 82 proteins were significantly different between SLE and HC. Among them, 14 had positive and 4 had negative correlations ($|r|>0.4$) with SLEDAI. We focused on C-type lectin domain family 7 member A (CLEC7A), which showed one of the strongest correlations with SLEDAI ($r=0.48$). CLEC7A levels were higher in patients with SLE compared to HC ($p< 0.001$) or to SSc ($p< 0.05$). CLEC7A levels were significantly higher in SLE patients with normal complement and negative anti ds-DNA antibodies compared to HC ($p< 0.001$). From the immune cell subset analysis, 19 subsets showed significant differences between patients with SLE and HC. Fourteen subsets, including activated follicular helper T (Tfh, $p< 0.001$) and type 17 peripheral helper T (Tph17, $p< 0.0001$), were significantly different between SLE patients with low disease activity (SLEDAI≤4) and HC. Furthermore, 6 out of 19 subsets, including activated Tfh ($r=0.46$) and Tph17 ($r=0.50$), correlated with CLEC7A levels ($|r|>0.4$). [Conclusion] CLEC7A could be a novel biomarker to monitor disease activity beyond the established immunological markers in SLE.

Th114. Signatures of T Cell Migratory Receptor Expression in Rheumatoid Arthritis Joints

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Abstract Text: Rheumatoid arthritis (RA) is an autoimmune disease characterized by T cell infiltration into the synovium. T cell migration into inflamed sites is controlled in part by expression of chemokine receptors that enable migration towards chemokines produced at sites of inflammation. Here, we characterized the expression of chemokine receptors on T cells that accumulate in synovial fluid and tissue of RA patients. T cells in synovial fluid are markedly enriched for expression of CCR2, CCR5, and CXCR6, three receptors that detect 'inflammatory' chemokines, compared to blood T cells. These receptors are often co-expressed and expressed separately from CCR7 and CXCR5. We further investigated these migratory programs by generating bulk RNA-seq data on T cell populations from synovial fluid and blood sorted on chemokine receptor expression. $CCR2/CCR5/CXCR6^+$ cells had distinct transcriptional signatures compared to $CCR7/CXCR5^+$ cells. $CCR2/CCR5/CXCR6^+$ T cells were enriched for transcriptomic signatures associated with T peripheral helper cells selectively in seropositive RA samples. We

utilized these datasets and ATAC-seq data to identify candidate transcription factors (TFs) that may regulate chemokine receptor expression, focusing on TFs that were upregulated in CCR2/CCR5/CXCR6$^+$ cells and had a likely binding site at a chemokine receptor locus. We tested these candidates in an arrayed CRISPR screen in human CD4 T cells and identified specific TFs that promoted or inhibited CCR2, CCR5, and CXCR6 expression on T cells. Here we have defined a migratory program of RA synovial T cells and identified candidate regulators that may influence T cell infiltration into inflamed synovium.

Th115. Spatial Proteomic and Transcriptomic Profiling of Lichen Planus

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Abstract Text: Lichen planus (LP) is a chronic autoimmune mucocutaneous disease characterized by pruritic skin lesions and painful mucosal ulcers. It is estimated to affect nearly 1 million people in the US. There are no FDA-approved therapies and current treatments are often ineffective in severe cases. To better understand the immune infiltration of lichen planus, we performed single cell spatial proteomics and spatial transcriptomics on biopsy specimens of LP with comparisons to other inflammatory skin diseases and healthy control skin. We performed multiplex ion beam imaging time-of-flight (MIBI-TOF) on archived biopsies and identified 26 unique cell populations, revealing a unique structured immune cell composition for each inflammatory skin disease. LP had the greatest number of regulatory T cells and CD8$^+$ memory T cells. Nearest neighbor spatial analysis revealed that CD11c$^+$ cells are the closest interacting cells with keratinocytes along the dermal epidermal junction. Next, we performed spatial transcriptomics using NanoString Digital Spatial Profiling to interrogate gene expression within CD4 T cells, CD8 T cells, and keratinocytes in a spatially resolved manner. Both T lymphocytes and keratinocytes express mixed inflammatory patterns in lichen planus. For example, keratinocytes have increased expression of type 1 interferon signaling, neuroinflammation signaling, Th1 and Th2 inflammatory pathway activation. Taken together, we have demonstrated a unique structured immune architecture in LP using both spatial proteomic and spatial transcriptomic approaches that may inform future therapeutic targets.

Th116. SPP1$^+$ Macrophages Are Pro-inflammatory and Pro-glycolytic in Rheumatoid Arthritis

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Abstract Text: Aim: Osteopontin/secreted phosphoprotein 1 (SPP1) plays an important role in inflammation and osteoclastogenesis. We previously identified enrichment of SPP1$^+$ synovial-tissue macrophages (STMs) in rheumatoid arthritis (RA) compared to healthy control (HC), correlating with clinical parameters with distinct glycolytic signatures. Methods: Blood obtained from HC and RA donors, CD14$^+$ monocytes isolated, and differentiated either in the presence of M-CSF (50ng/ml) or Ca2$^+$(2.5 mM) for 7 days to derive M0 or SPP1-expressing macrophages respectively. Inflammatory and metabolic outputs measured by RT-PCR, ELISA and Seahorse-XFE technology. Results: SPP1-expressing macrophages display increased gene expression of SPP1 and secrete osteopontin in high concentrations compared to M0, an effect that is greater in SPP1-expressing macrophages derived from RA patients compared to HC. SPP1-expressing macrophages also display increased gene expression of key pro-inflammatory mediators IL-6, TNFa, IL-1B and IL-8 and glycolytic genes HIF1a and HK2 compared to M0. Importantly this effect is more pronounced in RA vs HC indicating priming in the RA periphery. Mounting evidence indicates that altered metabolic pathways play a crucial role in directing cellular functions. To assess the metabolic mechanisms that underpin SPP1$^+$ macrophage function we utilized the seahorse analyzer. SPP1-expressing macrophages display decreased baseline oxidative phosphorylation and maximal respiratory capacity compared to HC/RA M0 while concomitantly skewing the ECAR/OCR ratio in favor of glycolysis indicating that SPP1-expressing RA macrophages rely heavily on glycolysis. Conclusions: This study demonstrates that RA SPP1-expressing

macrophages are metabolically re-programmed for sustained induction of pro-inflammatory responses. This may represent a potential pathway for therapeutic targeting early in disease.

Th117. Synovial Expression Levels of PD-1, the Target of Rosnilimab, Correlate with Disease Activity and Persist Across Disease Stages and Lines of Therapy in Rheumatoid Arthritis

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Abstract Text: Rosnilimab is an investigational PD-1 agonist that aims to regulate T cell activity for the treatment of inflammatory diseases, including rheumatoid arthritis, through depletion of PD-1high T cells and agonism of the PD-1 co-inhibitory receptor. In RA synovium, approximately 80% of T cells are PD-1$^+$ with enrichment of CXCL13 secreting PD-1high T follicular (Tfh)/peripheral helper (Tph) cells, which drive B cell differentiation to autoantibody-producing plasma cells. Synovial PD-1-associated genes from three published RA cohorts were evaluated for potential target populations for rosnilimab therapy: (1) treatment-naive early RA; (2) conventional synthetic disease-modifying antirheumatic drugs (csDMARD)-inadequate responders (IRs); and (3) anti-tumor necrosis factor (TNF)-IRs. RA synovium PD-1 expression was characterized across all T cell subtypes annotated from the Accelerating Medicines Partnership (AMP) single-cell data, while bulk transcript expression was assessed in all three cohorts. Tfh/Tph cells demonstrated the highest PD-1 expression compared with minimal to low PD-1 expression in naive and regulatory T cells, respectively. Across all cohorts, PD-1 and CXCL13 transcript levels were positively correlated with disease activity at baseline. T cell activation markers and CXCL13 expression correlated with CD3 expression across disease stages, indicating synovial T cells maintained an activated Tph-like phenotype. Irrespective of treatment, a correlation of PD-1 and CXCL13 expression with disease activity persisted, suggesting the importance of PD-1 in disease pathogenesis and a rationale for exploring rosnilimab's therapeutic potential. This will be evaluated in the RENOIR study, an ongoing Phase 2 trial of rosnilimab in moderate-to-severe RA patients (NCT06041269).

Th118. T Follicular Helper 1 Cells in Blood Potentially Mirror Salivary Gland-infiltrating T Cells in Sjögren's Disease

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Abstract Text: Sjögren's disease (SjD) is characterized by inflammation within the labial salivary glands (LSG) and systemic autoimmunity. However, the mechanisms linking blood and tissue immune responses remain poorly defined. In this study, we examined the immunological profile of circulating CD4$^+$ T cell subsets in SjD, focusing on Tfh1 cells that resemble LSG-infiltrating cells. Using multicolor flow cytometry, we found that CXCR3$^+$CXCR5$^+$ Tfh1 cells were significantly elevated in SjD than in healthy controls, both in circulation and in the LSG, with PD-1$^+$ICOS$^+$ Tfh1 cells correlating positively with antinuclear and anti-SS-A/B antibody titers. Conversely, while CXCR3$^+$CXCR5$^-$ Th1 cells were increased in the LSG, their frequency was diminished in circulation. TCR sequencing revealed notable T cell repertoire overlap between circulating Tfh1 cells and LSG T cells, with high similarities. Further analysis of the LSG microenvironment in SjD patients showed upregulation of IL-6, IL-12, IL-21, and TGF-β. Specifically, TGF-β in conjunction with TCR stimulation, promoted Tfh1 cell differentiation, driving IL-2, TNF-α, and IL-21 production and facilitating differentiation toward CD19$^+$CD38$^+$ B cells. Our findings suggest that Tfh1 cells in circulation may mirror the inflammatory and autoimmune activity in the LSG of SjD patients, with TGF-β playing a central role in driving disease-related Tfh1 cell differentiation. This study underscores the potential of Tfh1 cells as

biomarkers and therapeutic targets in SjD, warranting further investigation.

Th119. Targeting Aryl Hydrocarbon Receptor Functionally Restores Tolerogenic Dendritic Cells Derived from Patients with Multiple Sclerosis

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Abstract Text: Multiple sclerosis (MS) is a chronic disease characterized by dysregulated self-reactive immune responses that damage the neurons' myelin sheath, leading to progressive disability. The primary therapeutic option, immunosuppressants, inhibits pathogenic anti-myelin responses but depresses the immune system. Antigen-specific monocyte-derived autologous tolerogenic dendritic cells (tolDCs) offer alternative therapeutic approaches to restore tolerance to autoantigens without causing generalized immunosuppression. However, immune dysregulation in MS could impact the properties of the monocytes used as starting material for this cell therapy. Here, we characterized $CD14^+$ monocytes, mature dendritic cells, and vitamin D3–tolDCs (VitD3-tolDCs) from active, treatment-naive MS patients and healthy donors (HDs). Using multiomics, we identified a switch in these cell types toward proinflammatory features characterized by alterations in the aryl hydrocarbon receptor (AhR) and NF-κB pathways. MS patient–derived VitD3-tolDCs showed reduced tolerogenic properties compared with those from HDs, which were fully restored through direct AhR agonism and by use of *in vivo* or *in vitro* dimethyl fumarate (DMF) supplementation. Additionally, in the experimental autoimmune encephalomyelitis mouse model, combined therapy of DMF and VitD3-tolDCs was more efficient than monotherapies in reducing the clinical score of mice. We propose that a combined therapy with DMF and VitD3-tolDCs offers enhanced therapeutic potential in treating MS.

Th120. Targeting T Cell-myeloid Interactions in Immune Checkpoint Inhibitor-induced Myocarditis for Therapeutic Development

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Abstract Text: Immune checkpoint inhibitors (ICIs) have revolutionized cancer medicine by blocking inhibitory proteins expressed on T cells, such as PD-1 and CTLA-4, enabling T cells to attack tumors. However, approximately 1% of patients treated with ICIs develop myocarditis, which has a nearly 50% fatality rate. Current treatment strategies, hampered by a limited understanding of ICI myocarditis development, have minimal effectiveness and involve the use of steroids, which may compromise anti-tumor immunity. Identifying the cardiac-specific cellular subsets and signaling pathways that contribute to ICI myocarditis can aid the development of effective therapies without impacting tumor control. We developed a novel mouse model to identify the cellular and molecular mediators of cardiac inflammation in ICI myocarditis by treating tumor-bearing A/J mice with anti-PD-1 and anti-CTLA-4 monoclonal antibodies. We performed multiplex spectral flow cytometry on cardiac leukocytes and peripheral blood of these mice as well as immunofluorescence and spatial transcriptomics on their paraffin-embedded cardiac tissues in situ to identify the cellular ecosystems and molecular receptor-ligand interactions that may be pathogenic in ICI myocarditis. We found increased activated cardiac $CD8^+$ $CD69^+$ T cells in the heart that co-localize with infiltrating inflammatory myeloid populations in these mice with ICI myocarditis. Applying the CellChat pipeline to our spatial transcriptomics data, we identified myeloid and T cell communication pathways in the CXCL signaling axis as possible targets for treating cardiac inflammation under checkpoint blockade. These studies suggest significant targetable signaling pathways for treating ICI myocarditis while enhancing our understanding of organ-specific immune biology during ICI exposure.

Th122. The Effects of Antimicrobial Peptide LL-37 on B Cells in Psoriatic Arthritis

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Abstract Text: Background: Antimicrobial peptides released from neutrophils are involved in the pathogenesis of psoriatic arthritis (PsA). Among them, LL-37 is known to induce cytokine production from dendritic cells and T cells, triggering inflammation. However, its effects on B cells are unknown. This study aims to elucidate the effects of the antimicrobial peptide LL-37, which increases in the synovial fluid of psoriatic arthritis, on B cells. Methods: 1) Using the public scRNA-seq database of synovium samples from patients with rheumatoid arthritis (RA) and PsA, gene expressions of B cell clusters were evaluated. 2) $CD20^{+}CD27^{+}$ memory B cells were collected by sorting from peripheral blood mononuclear cells, and cytokine production of TNF and IFNγ from B cells stimulated with LL-37 were examined by ELISA. Results: 1) A total of 202,203 cells were collected from synovium samples of 4 RA and 5 PsA patients. Among them, 4,249 cells were identified as B cells. Pathway analysis showed that inflammatory cytokine signaling pathways such as the IL-17 signaling pathway and the TNF signaling pathway were more detected in PsA. 2) When memory B cells were stimulated with BAFF and LL-37, TNF production was significantly enhanced compared to the control group. No difference was observed in IFNγ production. Conclusion: Antimicrobial peptides such as LL-37 might contribute to the production of inflammatory cytokines from B cells. We are now investigating what signaling pathways are activated using bulk RNA-seq.

Th123. The Immune Profile of Circulating Autoreactive CD4 T Cells Is Imprinted Through Tissue Activation During Autoimmune Liver Diseases

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Abstract Text: Purpose: Autoimmune liver diseases (AILD) are immune-mediated disorders with CD4 T cell dysregulation, but the link between circulating self-antigen-specific CD4 T cells and the targeted tissue is still

unclear. We hypothesized that circulating self-antigen-specific CD4 T cells contain dominant hepatic CD4 T cell clonotypes and represent liver-derived autoreactive CD4 T cells. Methods: Self-antigen-specific CD4 T cells were isolated using CD154 upregulation following peptide stimulation, or with synthetized Sepsecs-peptide-loaded fluorescent-labeled MHCII-tetramers. Single-cell RNA-sequencing and TCR-sequencing were performed to investigate autoreactive CD4 T cell transcriptomic profile and clonotypes. A mouse model based on inducible expression of hepatocellular model antigen was designed to study the initiation and immuno-regulation of local antigen-specific immune response in the liver. Results: Circulating self-antigen-specific CD4 T cells from AILD patients exhibit a specific B-helper and immuno-exhausted transcriptional profile, which is conserved for different autoantigens but distinct from foreign antigen specificities. In the blood, the PD-1$^+$TIGIT$^+$HLA-DR$^+$ CD4 T cell subset shows a similar transcriptional signature to self-antigen-specific CD4 T cells and tissue-resident T cells, and contains highly expanded clones in the liver. In the mouse model, antigen-specific CD4 T cells acquire an immuno-exhausted transcriptional profile following their accumulation in the liver after local antigen reactivity, which is controlled by the immune checkpoint molecules PD-1 and CTLA-4. Conclusion: Our findings reveal that autoreactive CD4 T cells in the blood of AILD patients may be imprinted by chronic self-antigen stimulation in the liver environment. Thus, deciphering circulating PD-1$^+$TIGIT$^+$HLA-DR$^+$ CD4 T cells could provide autoimmune biomarkers and therapies.

Th192. Therapeutic Treatment with PLGA Nanoparticles Containing a Single CD4$^+$ Epitope Reverses CD8$^+$ T Cell-induced Type I Diabetes Disease Progression

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Abstract Text: Type 1 diabetes (T1D) is characterized by T and B cell responses to pancreatic β islet cell proteins, insulitis, and β islet cell loss. While, tolerance induction to individual diabetogenic proteins, e.g., GAD65 or insulin, has failed to ameliorate T1D both in wildtype NOD mice and T1D patients, treatment of NOD mice with poly(lactic-co-glycolic acid) (PLGA) nanoparticles (COUR CNPs) containing recombinant insulin, GAD65, and chromogranin A proteins (CNP-T1D) inhibited antigen (Ag)-specific T cell responses and disease progression. The ability of therapy to regulate CD8$^+$ T cell activity and the subsequent disease progression due to CD8$^+$ T cells was unknown. Our data show that treatment of NOD mice with TIMPs containing whole diabetogenic antigens results in an increased number of CD4$^+$ and CD8$^+$ T cells expressing regulatory markers and a decrease in disease incidence. While these findings show that Ag-specific treatment can modulate CD8$^+$ T cells, these effects may or may not require CD4$^+$ Tregs. Therefore, we sought to determine if treatment of mice with TIMPs containing only a single CD4$^+$ T cell peptide epitope within the same protein could result in modulated CD8$^+$ T cell function. Utilizing the OTI CD8$^+$ T cell transfer into RIP-mOVA mouse model of T1D, mice were treatment with unloaded TIMP vs TIMP-OVA323, i.e., a dominant CD4$^+$ T cell epitope, when mice had an average blood sugar of >225 mg/dL. The data show that TIMP-OVA323 treatment restored my to euglycemia for ~9 weeks in a Treg-dependent manner, and anti-CD25 treatment block tolerance

Th193. Application of HiFiBiO Drug Intelligence Science (DIS®) Translational Platform to Guide the Clinical Development of HFB200604, a Phase I BTLA Agonist Monoclonal Antibody

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Abstract Text: BTLA (B and T lymphocyte attenuator) is a co-inhibitory immune checkpoint expressed on B, T and dendritic cells. In contrast to blockade of individual cytokines, BTLA agonism has the potential to restore tolerance by impacting multiple pathogenic cell types and inflammatory cytokines. To date, BTLA agonistic antibodies have

failed to demonstrate the expected outcomes in patients with lupus and atopic dermatitis. Different factors might account for this, including inadequate antibody pharmacological profile and/or the selection of appropriate indications. Combining single cell technology and AI/ML algorithms, we applied our unique Drug Intelligence Science (DIS®) platform to discover and develop a best-in-class antibody, HFB200604, and to identify appropriate indications to address these challenges. HFB200604 is a differentiated BTLA agonist with optimized Fc-mediated agonism that significantly suppresses B cell proliferation, production of IFNγ, TNFα, and IL-17, and T cell activation and proliferation, both *in vitro* and *in vivo*. HFB200604 clinical evaluation is guided by DIS® single immune cell profiling of autoimmune diseases and focused on indications with reduced HVEM expression and BTLA signaling, where deregulated BTLA function can be restored through BTLA agonism. Based on the enrichment of a BTLA activation signature, we have prioritized autoimmune diseases where B and T cells exhibit lower levels of pathway activation. In conclusion, DIS® interrogation of patient datasets supports HFB200604 as a promising therapy to treat autoimmune diseases where BTLA suppressive functions are not fully engaged.

Th197. Terbinafine Associated Thrombotic Thrombocytopenic Purpura (TTP)

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Abstract Text: Thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic microangiopathy marked by severely reduced ADAMTS13 activity, leading to platelet-rich thrombi, thrombocytopenia, hemolytic anemia, and multi-organ damage. TTP can be acquired via an immune mediated process due to autoantibodies against ADAMTS13 or hereditary due to variations in the ADAMTS13 gene. Though various drugs have been linked to immune TTP, Terbinafine-induced cases are only anecdotally reported. We describe a case of immune TTP following Terbinafine use. A 39-year-old woman with rosacea presented with nausea, vomiting, and syncope during a 21-day Terbinafine course for tinea corporis. Exam showed petechiae, purpura, and pallor. Labs revealed hemoglobin 9 g/dL, platelets 20,000/uL, LDH 1,680 uL, undetectable haptoglobin, elevated reticulocyte count, and elevated creatinine. Coagulation studies and antiglobulin testing were normal. Blood smear revealed thrombocytopenia and schistocytes. Brain imaging showed subcentimeter intraprenchymal hemorrhages. ADAMTS13 activity was < 5% with antibody levels >90%. The patient underwent plasmapheresis and recovered fully with normalization of platelet levels after Terbinafine discontinuation. This case demonstrates immune-mediated TTP with multi-organ involvement following Terbinafine use, indicated by severe thrombocytopenia, hemolytic anemia, ADAMTS13 autoantibodies, and low ADAMTS13 activity - hallmarks of immune TTP. While drug-induced TTP is increasingly recognized, Terbinafine specific cases remain rare. Comprehensive pharmacovigilance and meta-analyses are needed to clarify drug associations and guide clinicians in identifying potential triggers.

Th198. Can the Gut Microbiota Trigger Autoimmune Responses Through Molecular Mimicry in Multiple Sclerosis? An *in vitro* Study

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Abstract Text: Multiple sclerosis (MS) is a chronic autoimmune disease characterized by demyelinating inflammatory lesions of the central nervous system (CNS). Mounting evidence suggests that the gut microbiota can trigger autoimmune responses, which may potentially contribute to MS onset through molecular mimicry mechanisms. In this *in vitro* study we investigated whether sterilized culture supernatants or cell pellets of bacteria isolated from MS patients activated 2d2 TCR-MOG reactive T cells through B cell- and dendritic cell- mediated antigen presentation. Bacterial pellets were used to prime antigen presenting cells and assess their ability to stimulate 2d2 CD4 T cells. Our results indicate that specific bacterial pellets promote Th1/Th17 polarization as evidenced by increased expression of T-bet and RORγt compared to myelin oligodendrocyte glycoprotein (MOG)

peptide control stimulation. In addition, certain bacteria increased CD44 expression in T cells, suggesting their activation. Some bacterial pellets also induced IL-17 production, suggesting their potential role in pro-inflammatory responses. B cells exposed to specific bacterial pellets and supernatants showed differential production of IL-23 and IL-10, highlighting the ability of gut microbiota to modulate inflammatory and immunoregulatory in MS. These *in vitro* findings support the hypothesis that gut microbiota may contribute to MS by promoting the activation of autoreactive T cells through molecular mimicry. Further studies are needed to confirm these results *in vivo* and elucidate the role of microbiota-immune interactions in triggering MS.

Tu107. Cryoglobulinemic Glomerulonephritis in the Setting of Multiple Myeloma: A Rare Clinical Presentation

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Abstract Text: Multiple myeloma (MM) is a plasma cell disorder producing monoclonal immunoglobulins, leading to skeletal destruction and renal failure. Cryoglobulinemia is a systemic inflammatory disorder that involves cryoglobulin deposition in small to medium sized vessels. In multiple myeloma, monoclonal immunoglobulins may behave as cold-induced cryoglobulins, causing vascular obstruction. We report a case of multiple myeloma manifesting as Type I cryoglobulinemic glomerulonephritis (T1C) with IgG2 kappa cryoglobulin (IgG2-K). A 74-year-old male with chronic kidney disease (CKD), hypertension, hyperlipidemia, rheumatoid arthritis (RA), monoclonal gammopathy of undetermined significance (MGUS), and seizure disorder presented with hypertensive urgency, shortness of breath, and severe renal dysfunction (Creatinine - 10). PET CT revealed lytic bone lesions and no evidence of renal obstruction. Renal biopsy confirmed Type I cryoglobulinemic vasculitis (IgG2-K). Due to multiple lytic lesions, renal involvement, and a history of MGUS, bone marrow biopsy was performed revealing MM. The patient was initiated on DaraCyBorD and later taken off dialysis with profound renal recovery. He continues with DaraCyBorD outpatient with clinical improvement MM that presents as T1C is rare, particularly in patients with pre-existing comorbidities. Diagnostic challenges included confounding conditions, such as MGUS-related renal failure and RA-associated renal amyloidosis. Additionally, the absence of classic cryoglobulinemia findings, such as arthralgia or purpura, further obscured the diagnosis. Although there have been multiple reports of IgGG3 cryoglobulinemic glomerulonephritis, IgGG2 CGN is exceptionally rare. This case underscores the importance of recognizing atypical presentations of multiple myeloma such as this one that manifested as T1C glomerulonephritis.

Tu108. TNFR2 and Its Immune-regulatory Role in Shaping Pathogenic B Cell Fate in Rheumatoid Arthritis

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Abstract Text: Background Pathogenic B cells, such as age-associated B cells (ABCs), are enriched in rheumatoid arthritis (RA) and other autoimmune diseases, correlating with increased disease activity. Targeting these subsets offers a promising approach for precision B cell therapies. Previous studies demonstrated TNFR2 expression in B cells, but its role in B cell function remains unclear. We hypothesize that TNFR2 promotes the inflammatory role of pathogenic B cells, contributing to RA progression and severity. Methods CITE-seq data from paired blood and synovium samples of RA patients(n=9) ,and PBMCs from blood(n=10) and synovial fluid (n=5) of RA patients were analyzed to evaluate TNFR2 gene and protein expression respectively . Purified B cells were stimulated under ABC-polarizing conditions in the presence or absence of TNFR2 agonists or antagonists.TNFR2 expression, B cell differentiation into plasma cells was analyzed by flow cytometry, cytokine secretion was measured by ELISA. Analysis was done using seuratV5, Flowjo, and GraphPad prism. Results CITE-seq revealed the highest expression of TNFR2 in ABCs. TNFR2 high B cells in the synovium but not in blood expressed lower levels of plasma cell

differentiation associated genes. There was a 4-fold increase of TNFR2 protein expression in synovial fluid B cells in RA patients compared blood B cells. TNFR2 agonist enhanced B cell TNF-α secretion (3-fold, $p< 0.05$) and inhibited B cell differentiation to plasmablasts (2-fold, $p< 0.05$). Conclusions TNFR2 promotes B cell antibody-independent inflammatory functions while inhibiting antibody-dependent functions. This novel finding underscores TNFR2 as a potential therapeutic target for precision B cell therapies.

Tu109. Transcriptomic Signatures of ANA$^+$ and ANA- B Cells Reveal Shifts from Active Disease to Remission in Systemic Lupus Erythematosus

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Abstract Text: In systemic lupus erythematosus (SLE), IgG antibodies target nuclear antigens (ANA). Healthy individuals (HC) possess ANA$^+$ mature B cells, without producing pathogenic antibodies. This study aimed to identify differential activation and regulatory mechanisms of ANA$^+$ B cells in patients with SLE and HC by single-cell RNA sequencing. We included patients with active disease (SLE-A) and in remission (SLE-R) to gain insight into the immune homeostasis of remission. We identified seven B cell clusters indicating distinct activation states within conventional B cell subsets. Compared to SLE-R and HC, SLE-A predominantly consisted of activated B cell and age-associated B cell (ABCs) clusters with fewer quiescent Naive and Marginal Zone B cells. SLE-R displayed both activated and quiescent clusters, but with fewer activated memory and ABCs compared to SLE-A, shifting to a B cell composition that is closer, but not identical, to HC. HC exhibited only quiescent clusters. Gene set enrichment analysis (GSEA) confirmed the presence of an interferon signature in both SLE-A and SLE-R. GSEA also revealed TNF-α signaling in activated clusters, which correlated inversely with interferon. The upregulation of the Fc receptor pathway in ANA$^+$ B cells from SLE-R and HCs, but not in SLE-A, suggests differential regulation. In patients with SLE, preferential accumulation of ANA$^+$ B cells within activated memory and ABCs was observed, supporting the notion that antigen-experienced ANA$^+$ B cells are selectively activated in SLE. These results provide new insights into the complex B cell landscape in SLE, highlighting potential targets for therapeutic intervention during different disease states.

Tu111. What Can Urine Single-cell Transcriptomics Teach Us About Kidney Immune Responses in Lupus Nephritis?

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Abstract Text: Immune cells are found in urine samples of lupus nephritis (LN) patients with active disease. We have previously showed, in a small-scale study, that gene expression in urine leukocytes in LN is highly similar to that in their kidney counterparts. Here, we generate single-cell RNA-sequencing (scRNA-seq) data from 140 LN patients, profiling both kidney and urine samples. We show that most leukocyte subsets found in LN kidneys can also be identified in urine. Using spatial transcriptomics data, we show that macrophage subsets that have a preference to localize to glomeruli are overrepresented in urine, suggesting that the glomeruli are a main gateway to urine for leukocytes. While the frequencies of leukocyte subsets in urine do not directly reflect those in kidney, we show that with simple linear modeling we can estimate kidney frequencies from urine frequencies with a median relative error of ~30% for most myeloid subsets. Furthermore, we show that the frequencies of specific urine leukocytes significantly correlate with the NIH Activity and Chronicity indices and the ISN class. Using linear regression models, we can estimate the Activity, Chronicity and ISN class from the frequencies of urine leukocytes with an AUC of 0.8-0.85. In addition, using this approach we can predict a 1-year treatment outcome with an AUC of 0.74. For comparison, models using the urine protein-to-creatine ratio (UPCR) yield an AUC of 0.54-0.68. Our work delineates the usefulness of urine profiling in monitoring kidney immune processes in LN nonintrusively, and provides a valuable resource to LN researchers.

Tu112. A Genome-wide CRISPR Screening Identifies Targets That Drive Tolerogenic Dendritic Cells

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Abstract Text: Tolerogenic dendritic cells (TolDCs) play a crucial role in maintaining immune tolerance and are promising candidates for treating autoimmune diseases. Despite its importance in the clinic, the key molecular pathways behind TolDC-mediated tolerance remain unclear. These cells are typically characterized by a functional status rather than a specific lineage marker, creating a challenge for identification of key modulators of tolerance. To overcome this, we identified a phenotypic biomarker representing TolDCs' functional status, allowing us to perform a genome-wide pooled CRISPR screen to discover new therapeutic targets that promote TolDC function. First, we confirmed that CD86 is consistently and significantly downregulated upon all tested conditions under treatment with different tolerizing agents (dexamethasone, vitamin D3 and rapamycin) in bone marrow-derived dendritic cells and human monocyte-derived dendritic cells. Furthermore, by using CD86 blocking Abs and CRISPR KO, we demonstrated the dependency of TolDC on CD86 inactivation to induce tolerance by suppressing T cell proliferation and activation, highlighting CD86 as a key screening read-out for assessing tolerogenic potential of DCs. Finally, through a genome-wide CRISPR-Cas9 screen, we sorted cells based on CD86 expression levels, which enabled us to identify and validate several genes that enhance the TolDC phenotype. We also uncovered critical molecular pathways necessary for producing highly effective TolDCs. Together, these projects are aiming at restoring immune tolerance in patients with IMIDs in line with our overall goal to induce the next wave of therapies that drive a cure.

Tu113. A Novel Biparatopic KLRG1 Antibody Selectively Depletes Effector Memory CD8$^+$ T Cells for Autoimmune Disease Treatment

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Abstract Text: Cytotoxic CD8$^+$ T cells contribute to multiple inflammatory and autoimmune diseases through tissue damage and destruction. KLRG1 (Killer cell Lectin-like Receptor G1) marks highly cytotoxic effector memory (TEM) and effector memory CD45RA$^+$ (TEMRA) CD8$^+$ T cell subsets that are expanded in the inflamed tissues or peripheral blood of autoimmune disease patients. Selective depletion of KLRG1$^+$ CD8$^+$ T cells is an attractive therapeutic approach that could offer lasting clinical benefits in selected autoimmune disease patients without global immune suppression. Using our proprietary single cell microfluidic platform, we identified a series of anti-human KLRG1 antibodies with high binding affinity and functional activity. Selected binding domains were used to design a biparatopic antibody, HFB04373, with single-digit nanomolar affinity to human and cynomolgus KLRG1 and capable of inducing more potent ADCC and ADCP of KLRG1$^+$ cells than a mono-specific benchmark *in vitro*. HFB04373 selectively depleted CD8$^+$ TEM and TEMRA subsets from PBMCs collected from healthy donors and autoimmune patients, sparing other T cells. HFB04373 also demonstrated potent and sustained dose-dependent depletion of KLRG1$^+$ KG1 cells *in vivo*. HiFiBiO's Drug Intelligent Science (DIS®) single-cell immune profiling platform provides insights into the abundance and activity of immune cell subsets in particular disease contexts, allowing precise targeting of pathogenic cell populations that drive autoimmunity in individual patients. Based on its potent pharmacological activity and favorable developability profile, HFB04373 is currently being developed as a novel immunotherapy for cytotoxic T cell-mediated autoimmune diseases coupled with a DIS®-enabled patient biomarker strategy.

Tu114. ALTB-268, a PSGL-1 Agonist Antibody, Ameliorated the Disease Severity in a Murine Model of Colitis and a Human-Mouse Xeno GVHD Model by Down-regulating T Effector Functions

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Abstract Text: Agonism of immune checkpoint regulators for the treatment of autoimmune and inflammatory diseases has high potential to become the next wave of immunotherapy. P-selectin glycoprotein ligand 1 (PSGL-1) is now known to function as an immune checkpoint regulator in T cells, in addition to its conventional role as an adhesion molecule involved in leukocyte trafficking. ALTB-268 is an immune checkpoint enhancer (ICE) that agonizes PSGL-1's regulatory role in T cell functions. *In vitro* studies showed ALTB-268 down regulates T cell activation, resulting in reduced upregulation of activation markers, diminished T cell proliferation and reduced secretion of inflammatory cytokines. The therapeutic potential of ALTB-268 agonism was evaluated in a humanized mouse model of xenogeneic GVHD (xGVHD) and a human PSGL-1 knock-in mouse model of DSS-induced colitis. In the human-mouse model of xGVHD, ALTB-268 improved animal survival, reduced GVHD severity score, and reduced human CD4$^+$, CD8$^+$ cells with increased 7-AAD$^+$ apoptotic T cells in the spleen of the ALTB-268 treatment group compared to isotype control. In the DSS-induced colitis model developed in human PSGL-1 KI mice, ALTB-268 ameliorated Disease Activity Index (DAI), reduced inflammatory cell infiltration in colon, and reduced TNFα and IFN-γ levels compared to isotype control. These results demonstrate the potential of Immune checkpoint enhancers (ICEs) in the treatment of autoimmune or inflammatory diseases and support the clinical development of ALTB-268 in T cell-mediated autoimmune and inflammatory diseases such as ulcerative colitis (UC) and GVHD. ALTB-268 is currently in an ongoing phase 2a study in UC (NCT06109441).

Tu115. Autoantibodies to PAD4 Associate with the Presence of Rheumatoid Arthritis, STAT3 Mutations, and Neutropenia in Patients with T-Large Granular Lymphocytic Leukemia

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Abstract Text: T-large granular lymphocytic leukemia (T-LGLL) is a lymphoproliferative disorder characterized by clonally expanded $CD8^+$ T cells enriched in somatic STAT3-activating mutations, neutropenia, and a strikingly high prevalence of rheumatoid arthritis (RA) compared to the general population. The peptidylarginine deiminase IV (PAD4) enzyme is strongly implicated in the pathogenesis of RA, and functions both as a generator of citrullinated autoantigens and as a direct target of autoantibodies in a subset of patients. Despite growing evidence for a central role of PAD4 in RA, the relationship between PAD4 and T-LGLL has not been explored. Here, we measured anti-PAD4 autoantibodies in well-characterized cohorts of patients with T-LGLL alone (n=95), T-LGLL with RA (T-LGLL/RA; n=94), and RA alone (n=380), and defined associations with neutropenia and STAT3 mutations. We found that patients with T-LGLL/RA were significantly more likely to have anti-PAD4 autoantibodies (60%) than patients with T-LGLL alone (2%; $p< 0.0001$), or RA alone (27%; $p< 0.0001$). Among anti-$PAD4^+$ patients, T-LGLL/RA patients had a higher median level of anti-PAD4 antibodies compared to RA patients (0.84 [IQR 0.70-1.01] vs. 0.59 [IQR 0.29-0.87] units; $p< 0.0001$). A larger proportion of anti-$PAD4^+$ T-LGLL/RA patients harbored activating STAT3 mutations in their peripheral blood mononuclear cells than anti-PAD4- patients (90% vs. 74%; p=0.047), especially the D661Y mutation (71% vs. 39%; p=0.005). Also, in T-LGLL/RA, anti-PAD4 levels were inversely correlated with absolute neutrophil count (Spearman's rho=-0.236; p=0.022). These data suggest a mechanistic link between humoral immunity to PAD4 and the presence of RA in the T-LGLL population.

Tu116. Base Editing Treg Cells to Avoid Teplizumab Binding to Enable Combination Therapy

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Abstract Text: Type 1 Diabetes (T1D) is an autoimmune condition characterized by immune destruction of beta cells leading to low insulin levels. Normally, immune tolerance results from depleting autoreactive immune cells and inducing regulatory immune cells, which suppress unwanted immune activation. We hypothesize that mimicking this process, inactivating effector T cells while enhancing regulatory T cells (Tregs), will lead to durable disease remission in T1D patients. Teplizumab, an FDA-approved treatment that slows the onset of T1D in high-risk individuals, is a humanized monoclonal anti-CD3 antibody which binds CD3ε and preferentially inhibits activated T cells. Thus, combining teplizumab with Treg is an attractive concept for restoring tolerance. However, Tregs are also targeted by teplizumab, making this therapy less synergistic. In this study, we used CRISPR-Cas9 base editors to edit CD3e and confer teplizumab resistance to Tregs. A pooled gRNA library spanning the extracellular domain of hCD3ε was used to identify targets that remove teplizumab binding while retaining the TCR-CD3 complex. The selected gRNAs were assessed for their ability to remove the Teplizumab epitope without loss of function. Successfully edited human T cells had no impairment in their responses to alloantigens *in vitro*. Their ability to induce xenogeneic graft-versus-host disease in NSG mice are being evaluated. We have shown it is feasible to edit CD3e removing Teplizumab epitope without negatively impacting T cell function. Future work will evaluate edited Tregs and teplizumab in appropriate T1D mouse models, to provide important pre-clinical proof of concept for combination tolerogenic therapy for T1D and other autoimmune diseases.

Tu117. BASTA, a Simple Whole Blood Assay for Measuring Beta-cell Antigen Specific CD4$^+$ T Cell Responses in Type 1 Diabetes

Stuart Mannering[1], Mathew Lacorcia[1], Pushpak Bhattercharjee[1], Abby Foster[1], Melinda Hardy[2], Jason Tye-Din[2], John Karas[3], John Wentworth[4] and Fergus Cameron[5]

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Abstract Text: Type 1 diabetes (T1D) is an autoimmune disease caused by the T cells mediated destruction of the insulin-producing beta-cells. Despite their clear pathogenic role the activity of beta cell antigen specific CD4$^+$ T cells cannot currently be routinely measured in a clinical setting. Here we describe the beta-cell antigen specific T cell assay – BASTA. BASTA is a simple whole blood assay that can detect human CD4$^+$ T cell responses to beta-cell antigens by measuring antigen-stimulated IL-2 production. BASTA is both more sensitive and specific than the CFSE-based Proliferation Assay. We used BASTA to identify the regions of preproinsulin which stimulated T cell responses specifically in blood from people with T1D. BASTA can be done with as little as 2-3 mL of blood. We found that effector memory CD4$^+$ T cells are the primary producers of IL-2 in response to preproinsulin peptides. We then evaluated responses to individual and pooled preproinsulin peptides in a cross-sectional study of paediatric subjects: without T1D, without T1D but with a first-degree relative with T1D, or diagnosed with T1D. In contrast to other preproinsulin peptides, full-length C-peptide (PI33-63) showed excellent specificity for T1D (AUC = 0.86). Further work has defined peptides from other beta cell proteins that elicit T1D specific CD4$^+$ T cell responses. Because of its simplicity, sensitivity and disease specificity we suggest that BASTA will be a useful tool for monitoring changes in beta-cell specific CD4$^+$ T cell responses both in research and clinical settings.

Tu118. CD4$^+$ T Cells Activated by IMQ Administration in IFNγKO Mice Ameliorate SLE Pathology via Suppression of Antibody-producing Cell Differentiation

Reona Tanimura, Yuya Kondo, Ryota Sato, Ryohei Nishino, Masaru Shimizu, Hiromitsu Asashima, Haruka Miki, Hiroto Tsuboi, Takayuki Sumida and Isao Matsumoto

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Abstract Text: Our previous study revealed that splenic IFNγ$^+$CD4$^+$ T cells were significantly increased after the induction of SLE model by IMQ treatment in C57BL/6 WT mice, thus we evaluated the pathogenic role of IFNγ$^+$CD4$^+$ T cells using IFNγKO (KO) mice. After induction of lupus model by IMQ, KO mice showed lower autoantibody titer and urinary protein, and amelioration of the deposition of immunocomplex in glomeruli compared with WT mice. Although Tfh and Tph cells in spleen showed no significant differences, IL-17$^+$CD4$^+$T cells were significantly increased and IL-10$^+$CD4$^+$ T cells were significantly decreased in KO mice. In addition, plasmablasts were significantly increased and plasma cells tended to be decreased in spleen of KO mice. Co-culture experiments of CD19$^+$ B cells of control mice with CD4$^+$ T cells of WT mice treated with IMQ or control WT mice were performed to evaluate B cell differentiation and IgG production, and revealed that plasmablasts and IgG levels in culture supernatant were significantly increased when using CD4$^+$ T cells of IMQ-treated mice. Moreover, differentiation of age-associated B cells and plasmablasts and IgG levels in culture supernatant were significantly decreased when CD19$^+$ B cells were co-cultured with CD4$^+$ T cells of IMQ-treated KO mice compared with IMQ-treated WT mice. These results suggest that IMQ-induced IFNγ$^+$CD4$^+$ T cells may be involved in autoantibody formation of SLE via enhanced differentiation of antibody-secreting cells.

Tu119. Characterization of S-1117, a Novel pan-IgG Protease Engineered by IMPACT Platform to Reduce Immunogenicity

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Abstract Text: Proteases derived from human pathogens can specifically cleave IgG antibodies. This unique trait represents a novel opportunity to use these molecules to treat IgG-mediated diseases. Due to the immunogenic nature of these proteases, their use as biologics is limited. S-1117 is a novel Fc-fused pan-IgG protease engineered using a proprietary machine learning enabled platform to reduce immunogenicity and augment manufacturability and stability while maintaining enzyme activity. *In vitro*, compared to the wildtype parental protease, S-1117 demonstrates reduced binding to pre-existing antibodies in plasma from healthy donors and reduced epitope display by MAPPs demonstrating removal of B and T cell epitopes, respectively. Furthermore, wildtype parental protease elicits CD4 T cell proliferation responses from 65% of healthy donors with a mean response index (RI) of 2.60 (magnitude of response over background signal). In comparison, S-1117 demonstrates a significant reduction in the number of responders to 20% and a 10-fold reduction in mean RI of 0.26 which are associated with reduced clinical immunogenicity. *In vivo*, repeated dosing of wildtype parental protease in C57BL/6 mice induced immediate hypersensitivity reactions, causing death in 60% (3/5) of mice and high ADA levels (>5ug/mL) in surviving mice. In contrast, all mice exposed to repeat doses of S-1117 survived without severe immune reactions and only 20% (1/5) of mice presented ADA levels. In summary, S-1117 is a novel pan-IgG protease engineered to remove B and T cell epitopes to overcome pre-existing immunogenicity and prevent *de novo* responses, while enhancing manufacturability and removing chemical liabilities without impacting enzyme activity.

Tu120. Characterizing Anca-specific B Cells in Anca-associated Vasculitis Patients

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Abstract Text: Anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) is a rare and severe autoimmune disease. ANCA-specific B cells have a central role in the pathophysiology of this disease, however current knowledge of their specific phenotypic markers and functional pathways is limited. Our aim is to characterize ANCA-specific B cells to gain insight into the pathogenesis of AAV. B cell receptor (BCR) repertoires and whole transcriptomes were analyzed with scRNA sequencing in B cells of 8 anti-MPO$^+$, 8 anti-PR3$^+$ and 5 HCs. 74 monoclonal antibodies (mAbs) were expressed of highly expanded and/or mutated B cells and their target antigens were screened with ANCA ELISAs. AAV patients had significantly increased plasmablasts (PBs) which exhibited large populations of clonally expanded B cells. The isotypes of the expanded B cells were predominantly IgA, and IgG1-2 and the somatic hypermutation (SHM) frequency was significantly increased for IgA. AAV patients had significantly increased light chain rearrangements and the lambda usage was overall higher. Differential gene expression analysis of the expanded B cell clones demonstrated upregulated genes involved in antibody production, B cell survival and migration in AAV versus HC. 10 ANCA-specific mAbs were identified, within 5 different patients. The ANCA$^+$ mAbs had varying SHM of 0–17%, clone sizes between 1-27, light chain rearrangements and were IgM, IgA, or IgG1/3, originating from varying B cell subtypes. In conclusion, our scRNA sequencing data reveals significant alterations in B cell populations and their functional characteristics, suggesting a potential ongoing

mucosal immune activation. Our characterization of ANCA-specific B cells and their dysregulated activation provides insight into the pathogenic mechanisms underlying AAV.

Tu121. Clinical Utility of Extractable Nuclear Antigens (ENA) Testing in Autoimmune Diseases: A One-year Analysis

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Abstract Text: We analyzed ENA results from 261 patients over a one-year period, comparing ANA results and clinical diagnoses to assess the utility of ENA testing. ENA was performed using EUROBlotOne(EUROIMMUN, Germany), and ANA with QUANTA-Lyser®2(Werfen, USA). Ro-52(23.4%) and SS-A(18.0%) showed the highest positivity rates, with speckled ANA patterns being the most common. Although SS-B antibodies were detected in only 4.6% of patients, all showed ANA titers of ≥1:320 with speckled pattern. SLE-specific antibodies, dsDNA and Sm, had 6.1%(16 patients) and 3.1%(8 patients) positivity. All Sm-positive patients exhibited SLE-associated symptoms, while 9 dsDNA-positive patients showed no SLE-associated symptoms, and 3 had low ANA titers. Nucleosome positivity(5.4%) was associated with SLE symptoms in 71% of cases. Homogeneous ANA patterns were predominant(78.6%), though rare patterns like PCNA were observed. The patient with the PCNA pattern also showed positive for PCNA in the ENA. Centromere B positivity(5.0%) correlated with SSc in 38.5% of cases, with high titers and characteristic ANA patterns. In contrast, Scl-70(1.5%) and PM-Scl(2.7%) positivity showed no association with SSc. RNP antibodies(10%) were associated to MCTD, with most cases showing high ANA titers (≥1:2560) and symptoms of rheumatic diseases. AMA positivity(5.0%) inconsistently correlated with primary biliary cholangitis, as 46.1% showed AMA ANA patterns, but only 2 patients were diagnosed with PBC. Among 35 patients with nonspecific symptoms such as cytopenia, myalgia, and FUO, ENA was negative in 51.4%, and 42.9% had negative ANA results or ANA titers of 1:40. Combining ENA results with ANA patterns and clinical context can support early patient management.

Tu122. Complex Pathogenesis of Axial Spondyloarthritis: Involvement of Type 1 Interferon Revealed by Single-cell RNA Sequencing

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Abstract Text: [Background] Spondyloarthritis (SpA) is a group of chronic inflammatory diseases that is characterized by primarily affecting the entheses and synovial joints. There is overlap among the diseases that comprise SpA and diversity of symptoms within the same disease, demanding stratification by immunophenotype. In this study, we aimed to reveal the relation between immunophenotype and axial lesions in SpA patients. [Methods] We recruited 77 patients with SpA, including psoriatic arthritis, axial SpA (axSpA), pustulotic arthro-osteitis. The scRNA-seq data on peripheral blood mononuclear cells and clinical information were integrally analysed. [Results] The presence of axial lesions was significantly associated with high abundance of plasmacytoid dendritic cells (pDCs, $P < 0.001$, OR = 2.28). Cell-cell interaction analysis revealed that pDCs had intensive signal to $CD8^+$ T cells and myeloid DCs (mDCs) in HLA-B27-positive cases. In contrast, HLA-B27-negative cases did not exhibit this interaction, and a significant association between disease activity and $CD4^+$ T cells characterized by high interferon-stimulated genes (ISG) was revealed ($P < 0.001$, OR = 2.01). pDCs were one of the major sources of type I

interferon and could stimulate CD4+ T cells. [Conclusion] scRNA-seq analysis demonstrated distinct pathological profiles of axSpA based on HLA-B27 status. In HLA-B27-positive patients, pDCs appeared to facilitate the activation of mDCs and CD8+ T cells, whereas in HLA-B27-negative patients, pDCs were suggested to produce type I interferon and stimulate CD4+ T cells. Stratifying patients by HLA-B27 status and targeting type I interferon in HLA-B27-negative cases could enhance the development of precision medicine.

Tu123. Contribution of Phosphodiesterase 1B to Neuropsychiatric Manifestations in Lupus-prone Mice via Microglial Activation

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Abstract Text: Background: Recent studies have shown that abnormal activation of microglia is involved in the pathogenesis of neuropsychiatric systemic lupus erythematosus (NPSLE). The aim of this study was to identify potential novel therapeutic targets for NPSLE. Methods: RNA sequencing (RNA-Seq) of microglia isolated from lupus-prone MRL/lpr mice and control strain MRL/MpJ mice was performed to explore candidate genes involved in microglial activation. We generated microglia-specific conditional knockout (cKO) mice to evaluate the function of the candidate gene in microglia. Gene expression of proinflammatory cytokines by real-time PCR and phagocytosis assays were performed in microglia from cKO mice. RNA-Seq and pathway analysis of cKO and control mice microglia were conducted to clarify the mechanism of altered expression of the candidate gene. An imiquimod-induced lupus model was used as an NPSLE model in cKO and control mice. The effects of an inhibitor of the candidate protein were evaluated in MRL/lpr mice via intracerebroventricular administration. Neurological manifestations were assessed by behavioral tests. Results: Among up-regulated genes in RNA-Seq, we focused on phosphodiesterase 1b (Pde1b), one of the top-fold changed genes. Gene expression of proinflammatory cytokines and phagocytosis were significantly suppressed in Pde1b cKO microglia. Gene Ontology analysis showed that the top five down-regulated pathways were related to the proinflammatory response. Hk1, a glycolytic enzyme, was involved in all these pathways. Pde1b cKO mice showed reduced imiquimod-induced behavioral abnormalities. Intracerebroventricular administration of the PDE1B inhibitor ameliorated behavioral alterations in MRL/lpr mice. Conclusion: Our data suggest that Pde1b could be a novel therapeutic target for NPSLE.

Tu124. Thymic Selection of Multiple T1D-Associated HLA Class II-Restricted Human T Cells

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Abstract Text: Type 1 diabetes (T1D) is a chronic autoimmune disease driven by autoreactive T cells that target insulin-producing beta cells. To investigate the thymic development and selection of such T cells, we used human immune system mice generated with human fetal hematopoietic stem cells and thymus tissue. We studied the development of six potentially diabetogenic T cell clones expressing HLA-DQ8 or HLA-DR4-restricted T cell receptors (TCRs) reactive to InsB9-23, InsC9-17, InsC10-18, InsC/IAPP2 hybrid insulin peptide (HIP), and GAD65555-567. Flow cytometric analysis revealed distinct thymic selection outcomes across these clones, with varying levels of partial deletion, regulatory T cell (Treg) conversion, or peripheral escape. Clone 5 and clone 20D11 T cells, reactive to InsB9-23, underwent efficient negative selection or Treg conversion, as previously reported, indicating antigen encounter during thymic development. Interestingly, the InsC9-17-reactive clone A5.5, but not the InsC10-18-reactive clone A1.9, generated Tregs, reflecting either differences in TCR affinity for the cognate peptide or in proteolysis products of the C-chain in thymic antigen-presenting cells. No Treg conversion was observed for the HIP-reactive clone A3.10 and the GAD65555-567-reactive clone R164, suggesting a lack of thymic antigen

presentation. Analysis of scRNA-seq and TCR sequencing data from thymic tissue grafts is underway for a more comprehensive analysis of these T cell clones at different stages of development. These findings shed light on the interplay between thymic selection and peripheral escape in T1D pathogenesis, advancing our understanding of tolerance breakdown and autoreactive T cell development in humans.

Tu125. Defective Positive and Negative Selection of Thymocytes Derived from Type 1 Diabetic Patient Hematopoietic Cells

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Abstract Text: The possible role of defective thymic selection in driving Type 1 Diabetes (T1D) is unknown. Personalized Immune (PI) mice were established by transplanting thymectomized NSG mice with $CD34^+$ hematopoietic stem cells (HSCs) from T1D or healthy control (HC) donors, along with human fetal thymic tissue sharing the T1D-associated class II HLA risk allele. This allows *de novo* generation of a human immune system and exploration of thymocyte development in T1D immune systems. Following immune reconstitution, thymocytes were harvested and analyzed using single-cell 5' RNA sequencing (10X Genomics) and bulk TCR sequencing (Adaptive Biotechnologies) of various sorted maturation subsets. T cell receptor hydrophobicity, particularly in the mid-CDR3 (mCDR3) region, reflects autoreactivity. In HC thymocytes, average mCDR3 hydrophobicity increased during positive selection ($CD4^+$ $CD8^+$ $CD69^-$ to $CD69^+$ cells) and remained stable in mature CD4 single-positive (SP) Tconv cells, indicating effective positive and negative selection. In contrast, T1D thymocytes showed a smaller hydrophobicity shift during positive selection and increased hydrophobicity in CD4 SP Tconv cells, suggesting impaired selection processes. Single-cell RNA sequencing also revealed T1D-associated deficits in thymocyte development. Transcriptional profiling of the normal human thymus identified maturing thymocyte subsets, including clusters linked to positive (hs-DP-3) and negative (hs-Sig-4) selection. These clusters were statistically significantly reduced in T1D mice, consistent with defective selection. Pathway and gene set enrichment analyses revealed apoptotic features in these clusters, further supporting disrupted selection. These findings provide independent evidence, both physico-chemical and transcriptional, of defective T cell selection in T1D mice. Ongoing studies aim to further investigate these mechanisms.

Tu126. Development of a Novel Beta Cell Targeted Immune Suppressive PD-1 Bispecific Agonist to Treat Type 1 Diabetes

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Abstract Text: Type 1 Diabetes (T1D) is an autoimmune disease driven by autoreactive T cells that destroy pancreatic islet -cells. The success of immunosuppressive therapies to preserve pancreatic islets has so far been limited, either due to safety concerns or poor efficacy. Recently, immunomodulatory therapies have emerged, e.g Teplizumab, showing efficacy in delaying onset of T1D. However, developing a tissue-targeted therapeutic approach would be safer. We have developed a novel, targeted immune-suppressive PD-1 bispecific agonist called ImmTAAI (Immune Modulating Monoclonal-TCR Against Autoimmune Disease) that specifically binds -cells and suppress autoreactive T cells. The molecule is composed of an affinity-enhanced T cell receptor (TCR) specific for the peptide-HLA-A*02 complex of pre-pro-insulin PPI15-24 fused to a PD-1-agonist. PPI PD-1 agonist ImmTAAI co-

localized with ENTPD3-labeled -cells in "*ex vivo*" pancreas tissue slices of non-diabetic and diabetic donors. Treatment of pancreatic slices from a T1D diabetic donor at onset, resulted in an increase of T cell mobility within the islets suggesting PD-1 ImmTAAI-mediated reduction of -cell-T cell interaction. In *in vitro* experiments, -cell bound ImmTAAIs suppressed TCR signaling, increased T cell motility, inhibited cytokine secretion, protected -cell from autoreactive $CD8^+$ T cell and NK cell killing. The molecule does not compete with PD-L1 avoiding impact on natural immune tolerance. Finally, the molecule did not interfere with mixed lymphocyte reaction confirming its sparing systemic immunity. In conclusion, these features indicate that the tissue-targeted PPI PD-1 agonist ImmTAAI may be an attractive and novel approach to treat T1D, offering improved efficacy and safety over systemically acting therapeutics.

Tu127. Diminished V-set Domain-containing T Cell Activation inhibitor-1 (VTCN1) Positive Immune Cell Subpopulations in Generalized Active Vitiligo

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Abstract Text: Background: Vitiligo, a prevalent skin-disfiguring autoimmune disease, has prompted the exploration of immunomodulation as a crucial strategy for intervention. VTCN1 (V-set domain containing T cell activation inhibitor 1), a member of the B7 superfamily molecules, plays a pivotal role in negatively regulating T cell activation. Given its significance, it becomes imperative to investigate the immunophenotypic profile of VTCN1 in both the Blood and Skin of generalized active vitiligo patients. Methods: We recruited 25 generalized active vitiligo patients and 30 healthy controls to assess VTCN1 expression in blood for the immunophenotypic analysis. Additionally, 13 generalized active vitiligo patients, and 10 healthy controls were included to examine VTCN1 expression on different T cells from the skin. Furthermore, we monitored VTCN1 expression on THP1 cells and evaluated the impact of IFN-γ, TNF-α, and IL-10 treatment using western blot analysis. Results: Our findings indicated a noteworthy decrease in the percentage of VTCN1-positive CD4, CD8, and regulatory T cells (p=0.030, p=0.004, and p=0.012) in vitiligo patients. Intriguingly, we observed a significant increase in activated T cells among vitiligo patients and a substantial reduction in $CD8^+$ $CD69^+$ $VTCN1^+$ positive cells (p=0.001 and p=0.016). Moreover, $VTCN1^+$ dendritic cells were significantly diminished in vitiligo patients (p=0.030). Conclusions: This study unveiled a significant reduction in VTCN1-positive CD4, CD8, regulatory T cells, and dendritic cells in vitiligo patients, indicating a breakdown in tolerance. This breakdown may trigger autoreactive T cells, fostering an exacerbated response against healthy melanocytes, ultimately contributing to melanocyte destruction and advancing the progression of vitiligo.

Tu128. Discovery of a FcgRIIb agonist antibody for treatment of autoimmune and inflammatory diseases

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Abstract Text: Within the family of receptors that bind the Fc portion of IgG (FcgR), FcgRIIb is the only member that functions as an inhibitory receptor. Co-ligation of FcgRIIb with activating receptors induces inhibitory signaling from FcgRIIb that negatively regulates the activation signal. Mice deficient in FcgRIIb have enhanced antibody response, increased IgE induced anaphylactic reactions, hypersensitivity to collagen-induced arthritis, and spontaneous development of lupus-like symptoms. Therapeutic targeting of FcgRIIb has proven challenging due to the 96% sequence identity of the extracellular portions of FcgRIIa, an activating FcgR, and FcgRIIb. Discovering antibodies to

FcgRIIb that do not also bind and activate FcgRIIa, which results in an unwanted production of pro-inflammatory cytokines, has been difficult. Furthermore, accelerated clearance has been associated with the use of high affinity antibodies to FcgRIIb. To identify novel FcgRIIb selective antibodies, we employed naive semi-synthetic libraries. Clone selection was performed by phage/yeast display and validated in scFV-Fc format. Interestingly, we observed that none of our FcgRIIb-selective clones were able to induce SHIP-1 recruitment in a cell-based assay. We then hypothesized that geometry of FcgRIIb binding might play a role in promoting agonism and converted scFVs to full-length antibodies. Converted FcgRIIb clones in the format of antibodies were now capable of initiating downstream signaling from FcgRIIb, highlighting the importance of the geometry in agonizing FcgRIIb. One of the identified FcgRIIb agonist antibodies does not block immune complex binding, has a favorable PK profile, and reduces antigen specific antibody responses in a KLH immunization model.

Tu129. Discovery of novel IgA proteases for the treatment of autoimmune and inflammatory diseases

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Abstract Text: IgA is a multifunctional immunoglobin that is important for tolerance to commensal bacteria in the gut luminal space but is also plays a role in detecting and neutralizing harmful pathogens systemically and in the gut. IgA can also contribute to inappropriate immune activation and inflammation in autoimmunity. Particularly, galactose deficient (Gd)-IgA1 is the antigen for autoantibodies and immune complex (IC) formation that contributes to kidney dysfunction in IgA Nephropathy (IgAN), one of the most common primary glomerulonephritis and a frequent cause of end-stage renal disease. For this reason, methods of clearing IgA are an attractive approach to the treatment of autoimmune and inflammatory diseases. Previously, bacterial derived serine proteases have been shown to clear ICs from models of IgAN and human tissue samples, however serine proteases are large in molecular weight and proved difficult to develop into therapeutics. Conversely, a 60kDa cysteine protease isolated from commensal bacteria has been described, however never sequenced. We therefore searched the bacterial genome for secreted proteins and identified protease candidates that retain the capacity to cleave IgA1. We used our Impact Platform and evolutionary screening, to identify additional novel cysteine IgA proteases from a variety of human commensal bacteria. Herein, we show that discovered proteases are potent in cleaving IgA1 from healthy donor serum and retain activity on biochemically Gd-IgA1 and IgA1 from IgAN patient serum, representing a promising novel approach to eliminate IgA. Further protein engineering and optimization with our Impact Platform for the novel proteases is currently under way.

Tu130. Dissection of the Mechanisms That Drive Loss of B Cell Anergy During Development of Autoimmunity

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Abstract Text: INTRODUCTION: B cells play an essential role in T1D pathogenesis by priming autoreactive T cells; however, their specific function remains understudied. Upon leaving the bone marrow, mechanisms of peripheral tolerance render autoreactive B cells anergic. Anergy has been described in a subpopulation of naive CD27-/IgM-/IgD^{+} B cells, coined Naive IgD positive B cells (BND cells). We identified a novel subset of BND cells, termed BND2 cells, that exhibit elevated markers of activation, suggesting escape from anergy. We found BND2 cells are increased in the blood and pancreatic lymph node of T1D patients compared to controls. This project aims to identify mechanisms enabling BND2 cells to escape peripheral tolerance, compared to traditional BND cells, hereon referred

to as BND1 cells. METHODS: We leveraged cell culture and murine models of T1D to identify soluble factors contributing to BND2 cell development. Using single-cell RNA-sequencing and machine learning algorithms, we compared the physicochemical properties of the BCR from BND2 cells to those of BND1 and other B cell subsets. RESULTS: We found that synergism of IFNγ and TLR7 agonism is essential for converting BND1 cells into BND2 cells. Additionally, our machine learning model reliably identifies autoreactive B cell subsets based on physicochemical properties, uncovering potential differences in how BND2 cells interact with autoantigens compared to other B cell subsets. CONCLUSIONS: Our findings demonstrate that BND2 cells escape peripheral tolerance through mechanisms involving IFNγ and TLR7 signaling, coupled with unique physicochemical factors, which drives their activation and likely participation in autoimmunity.

Tu131. Distinct Patterns of Anti-cytokine Antibody Profiles in Patients with Systemic Lupus Erythematosus

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Abstract Text: Anti-cytokine antibodies (ACAs) are a group of autoantibodies that target interleukins, interferons and other cytokine subgroups. While typically associated with inborn errors of immunity and severe viral infections, this class of autoantibodies appear to also be enriched in patients with Systemic Lupus Erythematosus (SLE). Yet, which ACAs occur most frequently in SLE and how they relate to other autoantibodies seen in rheumatic diseases remains unknown. In our present work, we aim to characterize the prevalence of ACAs in patients with SLE compared to other autoimmune conditions and no autoimmune disease, and understand how ACAs correlate with other classic autoantibodies (AAbs) seen in autoimmunity. For our study, we analyzed the ACA and AAbprofile of 300 patients with SLE, 80 patients with Sjogren, 100 patients with Multiple Sclerosis and 400 individuals with no systemic autoimmunity. We used a custom-made bead-based immunoassay that can detect up to 85 different autoantibodies (40 ACAs and 45 AAbs) in the plasma of each individual in our cohort. Our data support previous observations of higher occurrence of antibodies against interferons in patients with SLE and highlight a relative abundance of additional ACA subgroups such as the pro-inflammatory interleukins IL-6 and IL-12. Moreover, we found unique correlations between certain SLE-associated autoantibodies and ACAs including correlations of anti-U1 snRNP antibodies with anti-IL-12 autoantibodies, and anti-Smith antibodies with anti-IL-22 autoantibodies. Our findings suggest that people with SLE have a distinct ACA profile compared to individuals with other or no autoimmune conditions and these might correlate with specific disease manifestations.

Tu132. Engineering Soluble Fcγ Receptors to Antagonize Autoantibodies & Treat Autoimmunity

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Abstract Text: Introduction: Autoantibodies drive autoimmune diseases by providing a link between the immune system and innocuous target cells. Tissues expressing self-antigens are opsonised by autoantibodies which in turn, facilitate the activation of Fc receptor-expressing innate immune cells and trigger the complement cascade. In this project, we aim to limit the inflammatory function of autoantibodies by engineering therapeutic proteins that occlude their Fc region. Hypothesis: Soluble proteins comprising the extracellular domain of Fcγ receptors will reduce antibody-mediated activation of innate immune cells by creating a competitive environment for engaging antibody Fc regions. Results: Using HER2 as a model target antigen, we have developed an *in vitro* culture system that mimics autoantibody-driven inflammatory responses. In this system, HER2-expressing SKBR3 cells are opsonised with a clinically-relevant anti-HER2 antibody (Herceptin) and subsequently co-cultured with Fc receptor-expressing

monocytes/macrophages. Upon co-culture, monocytes/macrophages upregulate co-stimulatory molecules and secrete significant quantities of pro-inflammatory cytokines including IL-1β, IL-6 and TNFα. We have also demonstrated that soluble Fcγ receptors are capable of binding Herceptin-opsonised SKBR3 cells in a dose-dependent manner. In these assays, His-tagged proteins comprising the high affinity CD64 (FcγRI) ectodomain bound opsonised SKBR3 cells significantly better than proteins comprising CD32a (FcγRIIa), CD32b/c (FcγRIIb/c) or CD16 (FcγRIII) ectodomains. Future directions: We will assess whether soluble Fcγ receptors inhibit the antibody-mediated activation of monocytes/macrophages, as well as NK cell-mediated lysis, upon co-culture with Herceptin-opsonised SKBR3 cells. We will also probe whether Fc occlusion using soluble Fcγ receptors affects triggering of the complement cascade.

Tu133. FoxP3 Lo T Cells Are Induced in Autoimmune Inflammation and Likely Represent Regulatory T Cells with Reduced Suppressive Capacity

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Abstract Text: Background: Human FoxP3 expressing T cells can be divided into FoxP3-Lo and FoxP3-Hi subsets. The function and role of FoxP3-Lo T cells remains unclear. We examined FoxP3-Lo T cells across patients with systemic sclerosis (SSc), IgG4-related disease (IgG4-RD), and sarcoidosis. Methods: We used intracellular spectral flow cytometry to examine circulating Treg subsets. We used bulk RNA sequencing of purified T cell populations to define the transcriptional differences between subsets. Purified Treg subsets were used for *in vitro* activation and suppression studies. Results: In SSc and IgG4-RD, two progressive autoimmune diseases, FoxP3-Lo T cells, which have previously been shown to have a non-suppressive phenotype, were preferentially expanded and correlate with disease severity, whereas FoxP3-Hi T cells were not. In contrast, patients with clinically stable sarcoidosis, showed expansion of both FoxP3-Lo and FoxP3-Hi compartments. Transcriptionally, CD127-Lo CD25-Lo T cells closely resemble their CD127-Lo CD25-Hi counterparts but with reduced expression of IKZF2, IL2R, FOXP3, CTLA4, and activation markers including CD30, CD70, and HLA-DR. Functionally, CD127-Lo CD25-Lo T cells can be further activated with prominent upregulation of CTLA4 and show an intermediately suppressive capacity. Conclusion: We show that FoxP3$^+$ T cells expressing low levels of FoxP3 are bona fide regulatory T cells but display reduced suppressive functionality. These cells are expanded in settings of chronic inflammation. The correlation of FoxP3-Lo T cells with disease severity in SSc and IgG4-RD suggests that these cells either have a pathogenic role or reflect a distorted Treg phenotype that is permissive to T cell driven inflammation.

Tu134. Functional Alterations of Regulatory T Cells in Elderly-onset Arthritis

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Abstract Text: [Objective] To clarify age-related alterations of regulatory T cells (Tregs) in rheumatoid arthritis (RA) patients and GPI-induced arthritis (GIA) mice. [Method] 1) RNA-seq of Tregs from aged and young healthy subjects (HC) and untreated RA patients was performed. 2) GIA was induced in young and old mice after Treg depletion, and the arthritis scores were compared. 3) Tregs were isolated from young and aged GIA and naive mice, and their inhibitory functions were evaluated by suppression assay and cell metabolism assay. 4) CD4$^+$ T cells were isolated from inguinal lymph nodes of GIA and control young and aged mice, and single cell RNA-seq was performed to find

specific DEGs in Treg clusters. 5) Functional changes in young and aged Tregs induced by interferon (IFN)-β were evaluated by suppression assay and qPCR. [Results] 1) Aged Tregs were less activated than young ones in RA, and this difference was not detected in HC. In addition, pathway analysis of genes that varied between young and aged RA Tregs showed that IFN-related pathways were enriched. 2) The arthritis was significantly worsened only in the young mice. 3) Aged Tregs showed lower inhibitory function and OCR under GIA. 4) Treg clusters from aged mice showed enhanced type I IFN signaling. 5) Treatment with IFN-β resulted in decreased suppressive function of aged Tregs and increased expression of PD-1. [Conclusion] It was suggested that in elderly-onset arthritis, type I IFN signaling in aged Tregs was enhanced and PD-1 expression was elevated, leading to the impaired Treg function.

Tu135. Genetic, Transcriptomic and Proteomic Evidences Identify Targets with Higher Clinical Probability of Success

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Abstract Text: The low success rate of drugs in clinical trials across most therapeutic areas presents a significant challenge for pharmaceutical development. Previous studies and the Open Targets Platform have shown that human genetic evidence is a strong predictor of clinical trial success. However, the impact of multi-omics evidence on clinical trial outcomes remains less quantified. To address this, we compiled a comprehensive dataset of multi-omics target-disease pair associations, including 18,723 genetic associations (GWAS), 332,460 transcriptomic differential gene expressions (bulkRNA) across various tissues and cell types, and 96,283 plasma proteomic measurements from OLINK panels, covering 123 immunoscience diseases. We then calculated the probability of success (POS) of targets and target-indication pairs with or without multi-omic evidence. Our study reveal that targets supported by multi-omics evidence are significantly enriched in of approved targets and the have a higher probability of successful transition from phase I to approval (POS): confirming the importance of genetics (OR = 5.1 for approved targets and OR = 2.64 for POS), but also differential expression (OR = 5.4 for FDA-approved targets and OR = 2.68 for POS), and plasma protein (OR = 3.65 for POS). These results suggest that not only genetic but also transcriptomic and proteomic can improve clinical trial POS. Furthermore, we demonstrate that combining multi-omics evidence can result in better enrichment for approved targets (OR = 15.1). In conclusion, our data-driven, end-to-end target and drug indication positioning approach can accelerate the delivery of critical therapies to patients by accelerating clinical development.

Tu136. GPI-APD Kit with Veri-cells Control: A Comprehensive Solution for Studying GPI Anchor Protein Deficiencies

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Abstract Text: Research into GPI anchor protein deficiencies (APD), including paroxysmal nocturnal hemoglobinuria (PNH), often relies on custom or home-brew monoclonal antibody (mAb) cocktails, which can result in variability and inefficiency. Multicolor flow cytometry is critical for studying APD, including PNH, as it enables accurate identification and quantification of PNH clones across various cell lineages. In 2018, ICCS/ESCCA guidelines recommended standardized protocols for PNH assays, emphasizing the importance of reproducibility and accuracy. To address these needs, we introduce the GPI-APD Kit with Veri-Cells™ Control, a fully standardized flow

cytometry solution that ensures consistent and reliable assays for GPI-anchored protein deficiencies. The GPI-APD Kit includes the Veri-Cells positive control, which contains cells with undetectable GPI-anchored markers, ensuring reliable assay validation for PNH or other GPI-deficient cells. Additionally, the kit features FLAER conjugated to Spark Blue 488, a fluorochrome that highlights the absence of GPI-anchored surface markers, enhancing detection sensitivity. By incorporating a dried antibody assay format and a ready-to-use mAb cocktail, the kit improves staining reproducibility, minimizes preparation time, and reduces procedural errors. This comprehensive, guideline-compliant solution supports clinical-research-level investigations, offering laboratories a time-saving, robust, and standardized approach to studying GPI deficiencies. Keywords: GPI-anchored protein deficiency, paroxysmal nocturnal hemoglobinuria, flow cytometry, FLAER, Veri-Cells control

Tu137. Integrated Analysis of Serum Type I/II Interferons and RNA-Seq Identifies Interferon-mediated Cell Type-specific Effects and Prognostic Markers in SLE

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Abstract Text: Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease. Although interferon plays a central role in the pathogenesis of SLE, the specific effects of each type of interferon on immune cell types remain poorly understood. To uncover the cell type-specific effects of type I/II interferon, we performed an integrated analysis of RNA-seq and serum interferon measurements. We previously reported a bulk RNA-seq database for various immune cell types across multiple autoimmune diseases, including SLE. Serum levels of IFN-α and IFN-γ were measured using Simoa, an ultrasensitive digital ELISA technology, with stored serum samples from 112 SLE patients in this database. Differential gene expression and gene set enrichment analyses revealed that serum IFN-α levels were associated with oxidative phosphorylation and cell cycle pathways in T cells, particularly in Th1, ThA, and CD8 subsets, as well as in B cells. In contrast, serum IFN-γ levels were linked to antigen presentation pathways in monocytes and neutrophils. Focusing on 63 patients meeting the criteria for Lupus Low Disease Activity Status, those with elevated levels of both IFN-α and IFN-γ had the poorest prognosis ($p = 0.002$). Among all immune cell subsets, oxidative phosphorylation in ThA cells emerged as the most robust predictor of prognosis. In conclusion, a combined analysis of RNA-seq for various immune cell types and simultaneous measurement of serum IFN-α and IFN-γ uncovered the specific effects of each type of interferon. Gene expression analyses suggested that oxidative phosphorylation in ThA cells may serve as a novel therapeutic target to prevent disease flares.

Tu203. Epstein-Barr Virus Seropositivity Modulates the Immune Response to T Cell-Targeted Therapies in Type 1 Diabetes

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Abstract Text: Teplizumab, an anti-CD3 monoclonal antibody, is approved for delaying type 1 diabetes (T1D) onset

and modulating new-onset disease. Epstein-Barr virus (EBV) has been linked to autoimmune diseases, and our findings suggest that EBV serostatus influences the immune response to teplizumab and other biologic therapies. In the TN10 and AbATE trials, EBV-seropositive individuals experienced a more pronounced delay in T1D diagnosis and exhibited a greater C-peptide response compared to EBV-seronegative individuals. A similar trend was observed with anti-thymocyte globulin (ATG), which improved C-peptide responses in EBV-seropositive individuals, suggesting a broader immunomodulatory effect. At baseline in the TN10 trial, EBV-seropositive individuals had higher numbers of regulatory T cells (Tregs) and "partially exhausted" CD8+ T cells. Post-treatment, single-cell transcriptomics and functional assays revealed downregulation of NF-κB, T cell receptor, and mTOR signaling pathways, with greater impairment in adaptive immune cells, including B cells. Additionally, EBV-seropositive individuals displayed lower levels of anti-drug antibodies (ADA) in the AbATE. There was an increased expression of immune markers such as EOMES or KLRG1+ and TIGIT+ after teplizumab in the CD8 T cells. EBV-reactive T cells expanded following teplizumab, while T1D-specific T cells were curtailed. These findings suggest that EBV infection may alter immune signaling pathways, modulating T cell differentiation and enhancing the therapeutic response to teplizumab and ATG.

Tu215. STING and IFN-alpha/beta Receptor Are Required for Antigen-specific Apoptotic Cell-induced Peripheral Tolerance

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Abstract Text: Autoreactive T cell infiltration, tissue destruction, and spread epitope-specific T cell activation underly $CD4^+$ T cell-mediated autoimmune disease pathogenesis. While the successful use of antigen (Ag)-containing biodegradable poly(lactide-co-glycolide) nanoparticle, i.e. tolerogenic immune-modifying particles/Cour Nanoparticles (TIMP/CNPs) for the induction of Ag-specific $CD4^+$ T cell tolerance has been shown, the required signaling pathways remained elusive. Here, we identify that novel pathways are required for Ag-specific CNP-induced tolerance, and that these same pathways are required for apoptotic cell-induced Ag-specific $CD4^+$ T cell tolerance. The data show that myeloid cells phagocytose CNPs, undergo apoptosis, and release oxidized DNA (8-OHG). Subsequently, Ag-specific CNP treatment increases $FoxP3^+$ and $IL\text{-}10^+$ regulatory T cells via a stimulator of interferon genes (STING)/IFN-alpha/beta receptor (IFNAR)-dependent pathway. Treatment also regulates T cells specific for the spread epitopes associated with disease progression. Furthermore, induction of apoptosis via ECDI fixation, UV irradiation, X-ray irradiation, or culture of ECDI fixed RBCs with myeloid cells induces 8-OHG expression. Treatment of mice with these apoptotic cells, or Ag-coupled RBCs induces tolerance to the associated Ag and requires the host expression of STING/IFNAR pathways. Taken together, these results are the first to show that Ag-specific tolerance induced by the presence of apoptotic cells and by CNP-induced apoptosis requires the STING/IFNAR pathway.

W101. Methotrexate Reduces Post-vaccination Antigen-specific T Cell Response in Children with Juvenile Idiopathic Arthritis

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Abstract Text: Juvenile Idiopathic Arthritis (JIA) is a chronic, T cell-driven auto-immune disease that causes destructive, erosive joint inflammation. Its treatment involves immunosuppression with disease-modifying anti-

rheumatic drugs (DMARDs), which can potentially vaccine-induced immune responses and compromise protection against infection. Hence, there is a critical unmet medical need to delineate the DMARD effects on vaccine response to rationalise the vaccination strategy in these vulnerable patients. To address this unmet need, we interrogated the effects of methotrexate (MTX) on the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) specific T cell immune response in JIA patients (N=79) before and after receiving their first and second vaccine doses. Peripheral blood mononuclear cells (PBMCs) were stimulated with SARS-CoV-2 peptide pools for mass cytometry. Increased expression of activation-induced markers (AIM^+), CD154 and CD69, defined antigen-specific $CD4^+$ T cells. Median with interquartile ranges and Mann-Whitney U test were performed. Importantly, we found that with MTX treatment, the frequency of $CD4^+$ AIM^+ SARS-CoV-2 Spike-specific T cells was significantly reduced only after the second vaccination 0.60 (0.412-0.89)% versus 0.41 (0.26-0.79)% for no MTX versus MTX respectively (p=0.015). Moreover, for those on long-term MTX treatment, withholding MTX one week before the first and second vaccine doses significantly increased $CD4^+$ AIM^+ SARS-CoV-2 Spike-specific T cells: 0.27 (0.20-0.36)% versus 0.49 (0.35-1.20)% for MTX versus MTX withheld respectively (p=0.0063). MTX negatively impacts the strength of the antigen-specific $CD4^+$ T cell response after COVID-19 mRNA vaccination, withholding MTX can ameliorate this and increase the $CD4^+$ AIM^+ antigen-specific T cells. Translationally, this knowledge will help to refine the vaccine strategy in an evidence-based manner.

W102. Multi-parametric Interrogation for Systemic Immunological Perturbations in Patients with Juvenile Idiopathic Arthritis After MRNA Vaccination

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Abstract Text: Juvenile Idiopathic Arthritis (JIA) is a T cell-driven auto-immune disease affecting joints. In the Coronavirus Disease of 2019 (COVID-19) pandemic, mRNA vaccines elicit a robust immune response in healthy adolescents and young adults. There is an unmet need to determine if the COVID-19 mRNA vaccine has a systemic effect in children with JIA. We employed a high-dimensional approach, interrogating the peripheral blood mononuclear cells (PBMC) of 33 patients with JIA using a 43 markers mass cytometry panel. Quality check, batch-effect correction, flow-cytometry clustering by Self-Organising Maps, annotation and visualisation after t-distributed stochastic neighbour embedding dimensional reduction were done. Absolute differences in cell frequencies were expressed as mean differences with 95% confidence intervals (CI). Overall, there were no observable differences in the expression patterns of cytokines, checkpoint inhibitors, and inducible co-receptors with no significant changes in 42 immune subset frequencies across the immunomes at pre-vaccination, post-first dose, and post-second dose (ANOVA with Bonferroni correction). Specifically, the immune cell subsets that produced inflammatory cytokines (TNFα, IFNɣ, IL-2, IL-6, IL17A) had no significant mean frequency changes at post-dose 1 and 2 compared to pre-vaccination. The T regulatory cell frequencies showed no change at post-dose 1 (mean difference: -0.138 (95% CI: -0.636 to 0.360)%) and post-dose 2 (mean difference: -0.003 (95% CI: -0.458 to 0.452)%). Our results indicate the specificity of the mRNA vaccine in eliciting a controlled antigen-specific response without generalised immunological changes.

W113. A New Risk Gene Is Associated with Very Early Onset Inflammatory Bowel Disease and Autoimmune Hepatobiliary Disease: A Mechanistic Study Using Patient Organoids and Transgenic Models

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Abstract Text: Very early onset inflammatory bowel disease (VEO-IBD) and autoimmune hepatobiliary disease including primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH) are debilitating conditions with unclear etiologies. In a patient with VEO-IBD, PSC, celiac disease, hypothyroidism, and a strong family history of autoimmune disease, whole exome sequencing (WES) identified two rare, novel, pathogenic variants in CGNL1, which encodes an epithelial junctional protein. Both pathogenic variants were also present in the patient's younger sister with early onset and severe IBD and PSC-AIH. Furthermore, multiple relatives of the patient suffer from IBD, celiac disease, eosinophilic esophagitis, type-1 diabetes, and hypothyroidism. The RNA and protein levels of CGNL1 were diminished in the patient's intestinal epithelium compared to healthy individuals and other IBD patients. Intestinal epithelial organoids (IEOs) from the affected patient formed smaller 3D spheroids with altered cell composition and significantly impaired barrier function compared to those from healthy controls and other IBD patients. Electron microscopy demonstrated disrupted tight junctions and adherens junctions in the patient's IEOs. Similarly, impaired junctional complexes in intestinal epithelial cells and elevated intestinal permeability were present in Cgnl1$^{-/-}$ mice, which also showed increased susceptibility to colitis and C. rodentium infection. Single-cell analysis revealed diminished expression of CGNL1 in the intestinal and hepatic epithelial cells from patients with IBD or PSC/AIH, respectively. To summarize, we identified CGNL1 as a new risk gene that may contribute to VEO-IBD and autoimmune hepatobiliary disease by disrupting gut barrier integrity. This study expands our understanding of epithelial barrier impairment in the pathogenesis of VEO-IBD.

W114. Antigen-specific B Cells Cross-Present Citrullinated Proteins to Activate CD8 T Cells in Rheumatoid Arthritis

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Abstract Text: Dysregulated autoreactive CD8 T cell responses contribute to the pathogenesis of autoimmune diseases including rheumatoid arthritis (RA), a disease characterized by the development of anti-citrullinated protein antibodies (ACPA). In addition, polymorphisms in MHC class I are associated with ACPA$^+$ RA, including HLA-B*08 allele which harbors aspartic acid at position 9, suggesting that CD8 T cells can contribute to ACPA$^+$ RA pathogenesis. We previously identified clonally expanded cytotoxic granzyme B$^+$ CD8 T cells that target citrullinated antigens, and demonstrated that they are present in blood and synovium in RA; however, the underlying mechanisms by which these autoreactive CD8 T cells are activated to mediate joint tissue destruction in RA are unknown. Here, we demonstrate that oral mucosal breaks of bacteria provide an antigenic source of citrullinated proteins through neutrophil NETosis. We identify that citrullinated proteins are recognized and internalized by antigen-specific ACPA$^+$ B cells, and these B cells potently cross-present citrullinated proteins to activate anti-citrullinated protein specific CD8 T cells, which is dependent upon a Toll-like receptor (TLR) 4 signaling. We further demonstrate that citrullinated proteins stimulate B cells to secrete chemokines that attract cytotoxic CD8 T cells to form B-CD8 T cell aggregates, thereby facilitating antigen presentation to CD8 T cells in RA blood and synovium. Together, these findings suggest that antigen-specific B cells act as professional antigen-presenting cells that provide help to the activation of anti-citrullinated protein specific CD8 T cells to mediate their cytotoxicity that may lead to synovitis and joint destruction in ACPA$^+$ RA.

W115. Daratumumab Depletes Pathogenic Antibody-Secreting Cells, Reduces Type-I Interferon Activity and Improves T Cell Function in Systemic Lupus Erythematosus

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Abstract Text: Background: Antibody-secreting cells (ASCs), including long-lived plasma cells (LLPCs) produce pathogenic autoantibodies in systemic lupus erythematosus (SLE). LLPCs are resistant to immunosuppressive therapies and contribute to tissue damage. Daratumumab is a CD38-targeting monoclonal antibody that depletes ASCs and is approved for the treatment of multiple myeloma. Methods: This single-arm, open-label, phase 2 clinical trial evaluated the safety, efficacy, and immunological effects of daratumumab in patients with moderate to severe SLE. Ten participants were included that received eight weekly subcutaneous injections of 1800mg daratumumab and were followed for 36 weeks. The primary endpoint was the reduction in anti-dsDNA antibody levels. Immunological changes were assessed using using flow cytometry and single-cell RNA and immune cell receptor sequencing of memory B and T cells. Results: Daratumumab treatment led to a significant reduction in serum anti-dsDNA levels as well as vaccine-induced antibodies, indicating depletion of both short-lived ASC and LLPCs. This was associated with marked clinical improvement, reflected by a reduction of the median disease activity index SLEDAI-2K from 12 to 4 at week 12. Immunological analyses demonstrated a depletion of mature ASCs and a reduction in type-I interferon activity. TCR analyses indicate that $CD38^+$ memory T cells but instead $CD8^+$ T cell and regulatory T cells had reduced dysfunction. Discussion: Daratumumab induced rapid and sustained clinical improvements across organ systems in refractory patients SLE. The observed reductions in autoantibody-production, type I interferon activity and normalization of T cell functionality further support daratumumab as promising treatment option in SLE and potentially other systemic autoimmune diseases.

W116. Health-related Quality of Life in 85 Children with Primary Antibody Deficiencies in Mainland China: A Prospective Cohort Study

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Abstract Text: Objective The health-related quality of life (HRQoL) and treatment status of children with Primary antibody deficiencies (PADs) in mainland China remain under-researched. This study aims to assess the HRQoL of these children and identify associated factors. Method A prospective cohort study was conducted on children diagnosed with PAD and receiving medical care at our center from December 2017 to November 2022. Data were collected using demographic and treatment status questionnaires and the Parent-proxy Pediatric Quality of Life Inventory (PedsQL) 4.0 Generic Core Scale. Results A total of 85 PAD patients were included, with a mean age of 9.2 years (SD: 4.3); 79 (92.9%) were male, and 6 (7.1%) were female. Good treatment compliance with IVIG was observed in 61 (71.8%) patients. The median number of infections per patient in the past year was 3 (IQR = 1-7.5), and 18 (21.2%) patients had no chronic comorbidities. The average total HRQoL score was 69.46 (SD: 18.27), with the lowest subscale score in school functioning (55.61, SD: 19.98, $P < 0.001$). Social functioning scores were significantly higher than emotional functioning scores ($P = 0.023$). Multiple regression analysis revealed that poor treatment compliance ($P = 0.001$), chronic arthritis ($P = 0.018$), and chronic digestive disorders ($P = 0.028$) were significantly associated with lower HRQoL. Conclusions The HRQoL of children with PAD in mainland China is suboptimal. Enhancing treatment compliance and addressing chronic complications are critical for improving their quality of life. Comprehensive interventions involving social support, medical care, and psychological support are essential.

W117. Heterogeneity, Persistence, and Clonal Relationships of B Cell-helper T Cells in Lupus

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Abstract Text: T follicular helper (Tfh) cells are prototypical B cell helpers that drive high-affinity antibody production by regulating immune responses within germinal centers of secondary lymphoid organs. However, in chronic inflammation and autoimmune diseases, peripherally located T helper (Tph) cells expand. These cells share effector functions with Tfh, such as producing CXCL13 and IL-21, but lack CXCR5 expression and have reduced BCL6 levels. Although scRNA-seq has enhanced our understanding of B cell-helper T cell heterogeneity, the developmental relationship between Tfh and Tph remains unclear. Using TCR profiling, we describe longitudinal sharing of TCRs between Tph and Tfh cells in SLE patients and controls over a year (diagnosis, 6 months, 1 year). We demonstrate substantial TCR clonal overlap between Tph and Tfh cells, confirming a developmental link between these populations. We then performed scRNA-seq and TCR-seq on 3 SLE patients at 18 months post-enrollment to explore cellular heterogeneity and clonal relationships between sorted Tph and Tfh cells. Sorted Tph cells displayed greater phenotypic heterogeneity than Tfh cells, with both populations containing a cluster of $TOX^+CXCL13^+IL21^+$ cells that shared highly overlapping TCR repertoires. Using a pristane-induced lupus mouse model, we identified transcriptionally distinct B cell-helper T cell clusters in spleen and lung that shared TCR sequences. Ablating Bcl6 in $CD4^+$ T cells arrested Tfh development and disrupted broader B cell helper differentiation, including Tph cells. These findings demonstrate persistent clonal overlap between Tph and Tfh cells in SLE, suggesting a shared, Bcl6-dependent developmental history.

W118. HFB202501, a Novel Anti-PD-1 Antibody with Enhanced Effector Functions for Precision Depletion of Pathogenic T Cells in Autoimmune Diseases

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Abstract Text: Precision depletion of pathogenic immune cells is an attractive therapeutic strategy with the potential to reset the immune response and restore tolerance while retaining aspects of protective immunity. The immune checkpoint PD-1 has drawn interest as a therapeutic target in autoimmune and inflammatory diseases where it marks multiple pathogenic T cell subtypes. These include T peripheral and follicular helper (Tph and Tfh) cells, critical for the maturation and development of autoreactive B cells, which express very high levels of PD-1, and activated effector cells which contribute to inflammation and tissue damage and express lower levels of PD-1. Antibodies that agonize PD-1-expressing T cells and deplete PD-1(high) T cells have recently demonstrated clinical proof-of-concept in rheumatoid arthritis. We present here HFB202501, an anti-PD-1 agonist antibody engineered for enhanced depletion of $PD\text{-}1^+$ cells. HFB202501 shows more potent and deeper depletion of $PD\text{-}1^+$ T cells *in vitro* compared to clinical-stage benchmark antibodies. HFB202501 is able to deplete multiple $PD\text{-}1^+$ cell populations from human tonsils *in vitro* and is highly efficacious in a mouse model of lupus. Deep depletion of $PD\text{-}1^+$ cells has the potential for broad application in autoantibody-mediated autoimmune diseases and other indications where activated $PD\text{-}1^+$ effector cells play a pathogenic role. HFB202501 is progressing towards preclinical development with a biomarker strategy for identifying patients with expanded $PD\text{-}1^+$ cells populations.

W119. IL17-producing Follicular Helper T Cells Promote Germinal Center Autoreactivity in Systemic Lupus Erythematosus

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Abstract Text: Introduction Systemic lupus erythematosus (SLE) is an autoantibody-mediated disease affecting multiple organs. The frequency of circulating follicular helper T (Tfh) cells, which drive high-affinity antibody maturation, correlates with SLE activity. IL-17-producing T cells contribute to SLE development, and IL-17 inhibition improves disease in lupus-prone mice. We evaluated the role of IL17-producing Tfh (Tfh17) cells in SLE pathogenesis. Methods PBMCs from a cohort of SLE patients and healthy controls were analyzed. We generated lupus-prone mice in which IL17-producing Tfh cells could be fate-mapped and selectively depleted by diphtheria toxin (DT) administration (SLE Tfh17-DTR, Il17aCRE RosaLoxSTOPLox-YFP Cxcr5LoxSTOPLox-DTR Sle1$^{+/+}$Yaa^{+}). SLE Tfh17-FM (Il17aCRE RosaLoxSTOPLox-YFP Sle1$^{+/+}$Yaa^{+}), as well as Tfh17-FM and Tfh17-DTR mice (both Sle1$^{-/-}$Yaa^{-}) were used as controls. Results Tfh17 frequency was higher in SLE patients compared to healthy controls. Tfh17 cells peaked early (6 weeks) in SLE mice and could be selectively depleted by DT administration in the SLE Tfh17-DTR strain. Early Tfh17 deletion resulted in long-term reduction of splenic germinal center (GC)-B cells and plasmacells, and was associated with lower anti-dsDNA and anti-histone antibody levels at 20 weeks of life. Expression of IL17-receptor-A was higher on GC-B cells and plasmacells compared to follicular B cells in SLE mice. Bulk-RNAseq analysis of sorted B cell subsets exposed to IL-17a *in vitro* showed a higher fraction of differentially expressed genes in GC-B cells, with upregulation of a transcriptional program related to B cell survival and proliferation. Conclusions Tfh17 cells promote the formation of spontaneous germinal centers and the production of autoantibodies in SLE mice.

W120. Immune Modulation by Synthetic Progestins: Exploring Inflammatory Responses and Hormonal Interactions

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Abstract Text: Synthetic progestogens (progestins) used in hormonal contraceptives can have unintended side effects, including a heightened risk of autoimmune diseases. Research has shown that second-generation synthetic progestin levonorgestrel is linked with increased susceptibility to multiple sclerosis (MS), a CNS inflammatory disease. This study hypothesized that progestins, commonly used in contraceptives, may predispose individuals to inflammatory diseases, potentially exacerbating conditions such as MS. PBMCs from 24 healthy female and 6 healthy male participants were exposed to natural progesterone (P4) and various synthetic progestins at physiological doses: first-generation (norethindrone), second-generation (norgestrel, levonorgestrel), third-generation (norgestimate), and fourth-generation (drospirenone). Inflammatory cytokines were assessed using ELISA and quantitative PCR. Norgestrel, levonorgestrel, and drospirenone significantly increased IL-6 production in PBMCs from female subjects compared to P4, but this effect was not observed in males. The modulation of IL-6 by progestins, specifically levonorgestrel and drospirenone, was also validated at the mRNA level in a subset of samples. While P4 suppressed IL-6 production in PMA/ionomycin-stimulated PBMCs, confirming its anti-inflammatory ability, synthetic progestins failed to replicate this effect, underscoring their pro-inflammatory potential. Further investigation revealed differential effects on the expression of progesterone, androgen, and mineralocorticoid receptors in response to progestins and P4 in female PBMCs. In conclusion, our findings suggest that progestins have inflammatory potential with significant implications for conditions like MS as well as other autoimmune/inflammatory diseases. Further research with a larger cohort is currently underway to validate these findings and elucidate the mechanisms underlying these responses.

W121. Immune Repertoire Profiling in Type 1 Diabetes (T1D): Insights from BCR Analysis Across Multiple Tissues

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Abstract Text: T1D is an autoimmune disease driven by T cell–mediated β-cell destruction, with B cells contributing as antigen-presenting cells. Despite current diagnostic tools, there remains a critical need for biomarkers to track early disease progression and caputre individual variation in immune activity. B and T cell receptor (BCR and TCR) analysis provides an approach to monitor autoreactive immune acitivity, with potential to capture early disease dynamics and progression risk. This study analyzed BCR heavy-chain sequences from 57 donors from the Network for Pancreatic Organ Donors with Diabetes (nPOD), including spleen and pancreatic lymph node (pLN) tissues, to identify immune signatures distinguishing No Diabetes (ND, N=17), Autoantibody-positive (AAb^+, N=19), and T1D (N=21) states. Donors were 18–22 years old, with 49% female. CDR3 length analysis revealed significant demographic influences but no disease-specific differences. Mean CDR3 length was shorter in females($p=0.004$), decreased with age ($p< 0.001$), and shorter in African American individuals than European White donors ($p< 0.001$). V gene usage showed disease-associated biases. In the spleen, IGHV4-39 and IGHV4-59 were elevated in T1D, while IGHV3-1, IGHV3-49, and IGHV7-81 were reduced ($p< 0.05$). IGHV3-23 was higher in T1D than Aab^+. In the pLN, IGHV3-30, IGHV3-43 and IGHV3-49 were reduced in T1D vs. ND ($p< 0.05$). The selective enrichment and depletion of V genes may reflect disease-associated immune processes, highlighting B cell repertoire remodeling in T1D. Future work will focus on clonotype expansion and somatic hypermutation analysis, with emphasis on linking these patterns to antigen specificity, shared motifs, and public clones to better understand their relevance in disease.

W122. Immunity to Influenza B and Cross-reactivity to Pandemrix/pH1N1 in Narcolepsy

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Abstract Text: Background: Narcolepsy type 1 (NT1) is caused by 85-95% hypocretin (HCRT) neuron loss due to a combination of genetic and environmental risk factors and associated with DQA1*01:02/DQB1*06:02 (DQ0602, 98%) and T cell receptor (TCR) gene AJ24/AJ28/BV4-2. Pandemic influenza A H1N1 (pH1N1) and Pandemrix vaccination in 2009-2010 induced NT1 onset. Antigen specific T cell immune response in NT1 supports adaptive (auto)immunity against HCRT and Pandemrix/pH1N1. Despite this progress, the mechanism behind HCRT neuron loss and how the flu triggers a response against HCRT are unclear. Method: Anti-hemagglutination (HA) antibody was first measured using HA inhibition assay. Binding affinity of B/Victoria candidate peptides to DQ0602 was next tested using a competition binding assay. Antigen-DQ0602 restricted/reactive T cells were further isolated using tetramer DQ0602 and single-cell RNA sequenced with 10X. Lastly, TCR clones were transfected into Jurkat 76 for TCR activation by a certain peptide presented by DQ0602. Results: Two independent studies of our laboratory and a Chinese group have shown a higher HAI titer against B/Brisbane/60/2008 in NT1. We detected reactive $CD4^+$ T cells to a B/Victoria PB1 epitope that is homologous to Pandemrix/pH1N1 PB1 segment. In addition, we built a TCR repertoire a) restricted by HCRT and Pandemrix/pH1N1 at baseline peripheral blood mononuclear cells (PBMCs) ($n>430$) and b) from single-cell RNA sequencing cerebrospinal fluid (CSF) samples ($n>2,000$), and found the identical activated TCR clones in both baseline and cultured PBMCs. Conclusion: Our research showed influenza B plays a role in NT1 pathogenesis and potential cross-reactivity to PB1 between B/Victoria and Pandemrix/pH1N1.

W123. Increased CD56 Expression in Rheumatoid Arthritis Is Driven by 'Double Positive' CD56hiCD16hi NK Cells

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Abstract Text: CD56+ natural killer (NK) cell subsets can contribute to inflammation through inflammatory cytokines and cytotoxicity, and in inflammatory conditions such as rheumatoid arthritis (RA), 'double positive' CD56hiCD16hi NK cells have been reported. Because CD56 and CD16 are not exclusive to NK cells, others interpret these cells as CD56+ monocytes or dendritic cells. We thus aimed to comprehensively characterize CD56+ and CD16+ immune cells in RA. Using flow cytometry, we studied blood from RA patients and matched healthy donors (n = 16 each). We screened for CD56+ subpopulations (CD3–CD19–) by applying the SPADE (Spanning-tree Progression Analysis of Density-normalized Events) algorithm on an index patient (severe polyarticular RA). We quantified the resulting populations using conventional bi-axial gating, measuring mean fluorescence intensities (MFI) of cell surface molecules. We used SPADE on CD16+ subpopulations to further distinguish CD56+CD16+ NK from other CD16+ cells. RA patient blood contained a distinct 'double positive' CD56hiCD16hi subpopulation which was significantly expanded compared to healthy donors ($p < 0.001$). Unsupervised SPADE clustering showed additional populations significantly increased in RA, including CD56+CD16+ low-density neutrophils ($p = 0.003$) and dendritic cells ($p = 0.027$). In the index patient, CD16 increases were seen in CD11c+ dendritic cells and CD15+ monocytes. CD56+ cells are shifted towards CD56hiCD16hi 'double positive' NK cells, whereas the more modest CD56 and CD16 increases in dendritic cells suggest they are secondary to the inflammatory interferons and cytokines characteristic in RA.

W125. Increased TGFRI Signaling Within Fibroblasts Contributes to Chronic Intestinal Inflammation by Promoting Type 1 Immune Responses in Crohn Disease

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Abstract Text: BACKGROUND: Crohn's disease (CD) is a multifactorial disease involving an abnormal immune response to gut microbiota in genetically susceptible individuals, resulting in chronic inflammation. The dysregulation of TGFβ signaling is involved CD-associated immunopathogenesis. Increase in the expression of TGFβ receptor 1 (TGFBR1) on fibroblasts in CD-involved tissue was previously reported. However, the role of increased fibroblast-restricted TGFβR1 signaling in CD-associated inflammation remains unclear. Thus, the objective of this study was to evaluate how increased signaling through TGFRI within fibroblasts contributes to the pathogenesis in CD-relevant murine models of colitis. METHODS: Gain-of-function with a constitutively active (CA) TGFBR1 was conditionally induced in fibroblasts by a Col1a promoter-mediated Cre recombinase (TGFBR1ca-Fib) in mice during a chronic DSS colitis model. RESULTS: Analysis of publicly available scRNAseq data demonstrated an increase in TGFBR1 expression within inflammatory fibroblasts in CD. We observed that *in vivo* overexpression of TGFBR1 in intestinal fibroblasts leads to an abnormal tissue architecture, with increased collagen deposition in the submucosal and mucosal layers in murine colons. Transcriptomic analysis (bulkRNAseq) of TGFBR1ca-Fib mice colonic tissue demonstrated a significant enrichment of the genes and pathways involved in matrix deposition, as well as activation of immune response pathways with enrichment of adaptive immune response pathways. Furthermore, we demonstrated that hyperactivation of TGFβ signaling in fibroblasts prior to induction of chronic DSS colitis significantly aggravated intestinal inflammation, increasing mRNA expression of IFNγ. CONCLUSIONS: Collectively, our findings suggest that abnormal activation of TGFβR1 pathways within intestinal fibroblasts contributes to the type I inflammatory responses in CD.

W126. Intracytoplasmic TLRs and MyD88 in B Cells Subsets as Key Elements of Lupus Nephritis

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Abstract Text: We carried a cross-sectional study with 28 lupus nephritis (LN) and 26 non-renal active lupus patients, from which we isolated PBMCs to mark them for flow cytometry analysis of TLR7, 9 and MyD88 expression in total B cells ($CD19^+$), age-associated cells ($CD21^-$ $CD11c^+$ $T\text{-}bet^+$), classical memory ($CD27^+$), plasmablasts ($CD27^{hi}$ $CD38^{hi}$), transitional ($CD27^-$ $CD24^{hi}$ $CD38^{hi}$), non-classical memory ($CD27^-$ $CD24^+$ $CD38^-/_{lo}$), naive ($CD27^-$ $CD24^-$ IgD^+) and double negative (DN, $CD24^+$ $CD27^-$ IgD^-) B cells. Additionally, we measured 29 serum cytokine levels in a multiplex assay. After assessment of confounding variables with the G-test, we evaluated the relation of relative (%) and absolute (cells/mm3) number of cells expressing TLR7, TLR9 and MyD88, and their MFI with the development of LN with the Wald test. Finally, we selected a linear mixed model for renal activity with the highest relative quality, according to the Akaike information criterion. In order to avoid erroneous attribution of the results to treatment effects, analysis was delimited to subjects not receiving mycophenolic acid since a higher proportion of LN patients were under this treatment (60.7%, n=17 vs 30.8%, n=8, p=0.02). Higher MyD88 expression in transitional, naive, DN, classical and non-classical B cells related to renal activity. As well as increased TLR7 in naive, non-classical and DN B cells; and TLR9 in transitional cells. Eoxatin was the only cytokine significantly related to LN. Our final predictive model for LN includes TLR9 MFI in transitional B cells (OR 1.003, CI95% 1.001-1.01 p= 0.013) and eotaxin serum levels (OR 1.01 CI95% 1.003-1.03).

W127. Investigating Islet Autoreactive T and B Cells Using a Combined B Cell Antigen-specific and T Cell Activation-induced Molecule Assay

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Abstract Text: Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing beta cells in the pancreas. There are no measures of T1D disease activity in at-risk subjects to predict if and when an individual will progress to disease. This is, in part, attributed to our lack of understanding in how the landscapes of B and T cells evolve early in progressing and non-progressing stage 1 individuals. We show islet autoreactive (IAR) $CD4^+$ T cells are clonally expanded in stage 1 T1D and have diverse transcriptional phenotypes, with a proinflammatory effector phenotype being particularly enriched. To investigate B and T cell dynamics of early T1D, we developed a combined IAR B and T cell activation induced molecule (AIM) assay. To limit variability and minimize cell capture bias, we optimized key parameters including AIM choice (CD154 and CD137, $p< 0.01$ for $CD8^+$, $p< 0.05$ for $CD4^+$), positive control type (anti-CD3 and anti-CD28 titered to 10ng/ml and 1ug/ml, respectively), utility of enrichment (reduced sorting time to sequencing by 75%), MHC-I tetramer inclusion (not additive, $p< 0.05$), and IAR cell distribution (naive and memory subsets). With the ability to multiplex samples of different disease stage, we will interrogate IAR B and T cell subsets in stage 0 (pre-T1D), stage 1 (stable vs changing autoantibodies) and stage 2 or 3 (established T1D) using CITEseq, TCRseq, and BCRseq to identify key biomarkers of T1D disease activity and clonally expanded cell subsets to better understand immune signatures of early T1D that play a role in disease progression.

W128. Investigating the Mechanisms Underlying Regulatory T Cell Dysfunction in Ici-induced Colitis

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Abstract Text: While immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment, nearly 85% of patients experience autoimmune-like side effects known as immune-related adverse events (irAEs), with ICI-colitis being one of the most common. The mechanisms underlying irAEs remain poorly understood, particularly regarding the role of regulatory T cells (Tregs). Tregs are a subset of CD4 T cells essential for maintaining immune tolerance

and preventing autoimmunity. Recent findings from my work and others show a significant accumulation of proliferative, cytotoxic T cells in the colon. This is accompanied by upregulation of various type II interferon-associated genes. Specifically, Tregs exhibit increased expression of IL12RB2, CXCR3, and TBX21, suggesting that ICI-colitis is driven by Th1 responses. This distinguishes it from other colitis types, which are typically Th17-driven. Despite their activated phenotype and proliferation, Tregs are unable to control the autoimmunity occurring in the colon. We hypothesize that Treg suppression is dysfunctional in ICI-colitis. To dissect the transcriptomic changes occurring in Tregs in the context of ICI-colitis, we will employ single-cell RNA sequencing, along with *in vitro* studies. These will shed light on the origin, stability, and effect of Th1-programs on Tregs. Additionally, we will use spatial transcriptomics to examine Treg/CD8 T cell interactions in the colon. This will allow us to assess the colocalization of CD8 T cells and Tregs, determine whether suppression is occurring, and if CD8 T cells exhibit resistance to Treg-mediated suppression upon ICI treatment. Collectively, these studies will provide crucial insights into the mechanisms underlying ICI-colitis and other irAEs.

W129. Investigating the Role of the Chemokine Receptor, CCR1 in Cutaneous Lupus Erythematosus Pathogenesis

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Abstract Text: Investigating the role of the chemokine receptor, CCR1 in Cutaneous Lupus Erythematosus Pathogenesis Mridushi Daga, Saeed Shakiba, Jillian Richmond Introduction: Cutaneous Lupus Erythematosus (CLE) is an autoimmune disease that affects the skin, causing chronic inflammation. This inflammation leads to symptoms such as scaly rashes and lesions. Central to CLE pathogenesis is infiltration of leukocytes and proinflammatory chemokine production in the epidermis. These processes are regulated by various chemokine receptor axes. Studying these axes may help identify new targets for understanding and treating CLE. Results: Using spatial transcriptomics, we analyzed 17 chemokine receptors in human skin biopsies from CLE patients and identified CCR1 as the most significantly upregulated compared to healthy samples. Olink proteomics of interstitial skin fluid from suction blister biopsies further confirmed this, revealing increased levels of multiple CCR1 ligands (CCL3, CCL7, CCL8, CCL23) in lesional blister fluid compared to healthy controls. In a novel adoptive T cell transfer CLE mouse model, CCR1 knockout in T cells conferred partial protection against the development of severe disease. Additionally, monocytes, which are among the first cells to infiltrate the skin, also express CCR1. We plan to test the CCR1 antagonist BX471 in our mouse model, as it blocks both T cells and monocytes, potentially offering better protection against disease. Conclusions: The CCR1 chemokine axis plays a major role in recruiting disease-causing immune cells to the skin. Blocking this axis with antagonists may help protect against the development of CLE in our mouse model.

W130. Islet Autoreactive Regulatory and Conventional T Cells Have Distinct TCR Repertoires and Differ Transcriptionally from Foreign Antigen-reactive T Cells

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Abstract Text: Regulatory T cell (Treg) dysfunction has been previously implicated in the pathogenesis of type 1 diabetes (T1D). Despite growing knowledge of the conventional T cells (Tconv) that target the islet, little is known about islet autoreactive (IAR) Tregs. We established an AIM assay to isolate antigen-reactive Tregs and Tconv cells from three healthy individuals and three individuals with established T1D and analyzed them using multimodal 10X sequencing to determine whether IAR Tregs had unique features compared to those specific for foreign antigens. Antigen-reactive cells were isolated by stimulating PBMC with islet or CEFX microbial peptide pools, CD3/CD28 antibodies, or vehicle overnight. Each sample was then barcoded, combined, and enriched for $CD154^+$ and $CD137^+$ activated cells. Enriched cells were stained with CITE-seq and flow antibodies, sorted purified, and processed for 10X sequencing. After barcode demultiplexing, antigen-reactive cells were defined as CD154-$CD137^+CD25^+CD127_{low}$ for Tregs and $CD154^+CD69^+$ for Tconv cells using CITE-seq antibody expression. IAR Tregs were transcriptionally distinct from foreign antigen-reactive Tregs, with enrichment of cells expressing metabolism genes and fewer classic $HLA\text{-}DR^+CD39^+$ Tregs than foreign antigen-reactive Tregs. IAR Tconv cells differed from foreign antigen-reactive Tconvs with enrichment of cells with effector memory gene expression compared with cells enriched in interferon response gene expression in foreign antigen-reactive Tconv cells. Furthermore, IAR Treg and IAR Tconv had greater clonal expansion compared to foreign antigen-reactive cells. Notably, the TCR repertoires of Tregs and Tconv cells were distinct. This study identifies unique differences in IAR Tregs that may be important in T1D development.

W131. Islet-derived CD8 T Cells from Donors with T1D Express More Transcripts for Granzymes and Type 1 Interferon-induced Proteins Than Those from Donors Without Diabetes with Recognition of HLA-B Restricted Insulin Epitopes at T1D Onset

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Abstract Text: Islet-infiltrating T cell populations, along with other immune cell populations, are thought to be key effectors in T1D pathology resulting in destruction of insulin-producing beta cells. While there is strong evidence for the presence of islet-reactive CD8 T cells in the islets of donors with T1D, no functional phenotype is directly associated with islet-infiltrating, defined autoreactive CD8 T cells in human T1D. Isolated islets were received from 12 donors with T1D and 13 donors without diabetes. CD8 T cells from islets of both groups were analyzed by scRNA-Seq. Module scores of expressions of gene groups were generated and tested using a Wilcoxon rank sum-test. It was found that modules of transcripts of GZMA, GZMB, GZMH, and GZMM, and the type 1 interferon inducible genes including IFITM1 and IFITM3 were expressed at statistically higher levels in the islet-derived CD8 T cells from the donors with T1D as compared to those from donors without diabetes. From nPOD donor 6578, who died at onset of T1D without previous administration of exogenous insulin, nine TCR transductants from islet-derived CD8 T cells expressing granzyme transcripts were generated. Three of these recognized epitopes either within the A-chain or the B chain of insulin and each were restricted by the class I HLA-B alleles expressed by the donor. These data strongly support the presumed function of islet-infiltrating CD8 T cells in T1D as cytotoxic effectors and identify insulin epitopes and presentation by class I HLA-B alleles as important in T1D pathogenesis.

W132. Lupus Nexus: A Lupus Registry, Biorepository, and Data Exchange Platform to Foster Global Collaborations, Accelerate Research Innovation, and Advance Precision Medicine

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Abstract Text: Systemic lupus erythematosus (SLE) is a complex disease with significant unmet medical needs. Its heterogeneity and poorly understood etiology, pathogenesis, and subgroups hinder the development of targeted therapies. Advancing SLE research requires a centralized, longitudinally curated patient dataset linked with biosamples and molecular data. To meet this need, the Lupus Research Alliance launched Lupus Nexus (LNx)—a registry, biorepository, and data-exchange platform. Developed with input from over 100 stakeholders, including scientists, funders, and patients, LNx is anchored by the Lupus Landmark Study. This prospective, longitudinal study is currently enrolling up to 3,500 patients into four cohorts—new-onset SLE, extra-renal flare, active lupus nephritis, and prevalent SLE—tracking them over five years, generating extensive datasets paired with biospecimens. Clinical data include clinician- and patient-reported outcomes, medical and serologic histories, medication use, vaccinations, and other key information. To date, 221 participants have been enrolled from sites across the United States and Canada: 13% new-onset, 20% lupus nephritis, 25% extra-renal flare, and 42% prevalent. Participant demographics reflect the broader population, and over 12,000 biosample aliquots have been collected. Targeted genetic, transcriptomic, and proteomic assays will generate foundational scientific data to support preliminary research efforts and further utilization of LNx resources. Additional foundational datasets include quantitative auto-antibody measurements, microbiome metagenomics, and medication level assessments. LNx provides researchers access to biospecimens, robust datasets, and advanced computational tools while enabling participants to view their data and connect with others. This initiative aims to accelerate precision medicine in lupus, driving significant advances in diagnosis and treatment. www.lupusnexus.org

W133. Mechanisms Driving Dysregulated Germinal Center Dynamics in Systemic Lupus Erythematosus

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Abstract Text: Systemic lupus erythematosus (SLE) disrupts humoral immunity, promoting the development of autoreactive B cells within and outside lymph node (LN) follicles. This study combines spatial transcriptomics and multiplex imaging to dissect the immune landscape and transcriptomic features of LNs in SLE. Our analysis revealed prominent type I interferon (IFN) and plasma cell signatures in SLE compared to reactive control follicles. Cell deconvolution demonstrated that follicular T cell subsets are predominantly influenced by the type I IFN signature characteristic of SLE follicles.A closer look at T follicular helper (TFH) cells revealed significant alterations, including reduced numbers of Bcl6hi TFH cells and diminished representation of IL-4-producing TFH subsets alongside compromised IL-4 signaling in follicles. Additionally, the correlation between TFH and GC-B cell densities was lost in SLE. These changes coincided with a decline in Bcl6hi GC-B cells and an accumulation of extrafollicular CD19hiCD11chiTbethi age-associated B cells (ABCs), which are known for their autoreactivity. Additionally, SLE follicles exhibited increased IL-21$^+$ cell densities, indicative of a highly inflammatory microenvironment. These findings highlight the profound disruption of follicular immune regulation in SLE, likely driven by sustained inflammatory signals such as type I IFN and IL-21. This study provides critical insights into the cellular and molecular dynamics of SLE-affected LNs, identifying potential therapeutic avenues to restore immune balance and improve vaccine efficacy in SLE patients.

W134. Miller Fischer Syndrome as a Rare Mimic of Botulism

Jamal Obeid, Leen Khalife, Ahmed Souid and Vincent Calleo

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Abstract Text: Botulism presents with symmetric neurologic deficits including diplopia, facial weakness, and descending muscle weakness with normal mental status and absence of fever, but these symptoms overlap with a variety of conditions including immunologic neuropathies. We present the case of a 19-year-old male construction worker with no significant medical history who developed binocular diplopia, bilateral mydriasis, extremity weakness, and gait ataxia after eating poorly refrigerated sandwiches and daily exposure to roofing materials. On examination, he exhibited external ophthalmoplegia, symmetric extremity weakness, and normal reflexes in an otherwise afebrile and appropriate teenager. Basic labs and inflammatory markers were unremarkable. He was treated with botulinum antitoxin without improvement. Respiratory panel was positive for rhinovirus. Head imaging was normal. Lumbar puncture revealed albuminocytologic dissociation concerning for a post-viral autoimmune condition. He was treated with IVIG with marked improvement and was discharged home without need for rehabilitation. Send-out CSF studies were later positive for anti-GQ1b antibodies (387), Asialo-GM1 IgG/IgM antibodies (51), and an elevated cerebrospinal fluid IgG synthesis index consistent with Miller Fisher Syndrome (MFS), a rare variant of Guillain-Barré syndrome. This case highlights the importance of considering immunologic causes in addition to infectious and toxic exposures in healthy patients with new neurologic deficits. Intravenous immunoglobulins may be a suitable treatment in many of these diseases.

W135. MRT-6160, a VAV1-Directed Molecular Glue Degrader, Inhibits Disease Progression in a Preclinical Model of T/B-cell-Mediated Experimental Autoimmune Encephalomyelitis

Marisa Peluso, Adam Cartwright, Lucas Gyger, Foram Desai, Shailee Vora, Anna Kostikova, Laura Roditi, Xudong Wang, Peter Trenh, Katie May, Sophia Nguyen, Chris King, Daniel Lam, Xavi Lucas, Mary Zlotosch, Elisa Liardo, Daric Wible, Ilaria Lamberto, Bradley Demarco, Debora Bonenfant, Sharon Townson, Eswar Krishnan, Filip Janku, John Castle, Laura McAllister and Alison Paterson

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Abstract Text: VAV1 is a guanine nucleotide exchange factor critical for T cell receptor (TCR) and B cell receptor (BCR) signaling. Genetic loss of Vav1 confers diminished T/B-cell effector functions and attenuates autoimmune disease onset in preclinical models. MRT-6160 is a first-in-class MGD that specifically targets VAV1 for proteasome-mediated degradation and is currently under clinical development for immune-mediated conditions. MRT-6160 may provide therapeutic benefit across multiple T- and/or B cell-mediated conditions including neuroinflammatory diseases. MRT-6160-treated primary immune cells were TCR- or BCR-stimulated and assessed for various immune effector functions. MRT-6160 was then evaluated in a T/B-cell-mediated MOG protein immunization EAE mouse model. Mice were treated orally and daily from Day 9-20 with vehicle, fingolimod (3 mg/kg), or MRT-6160 (1 mg/kg); clinical scores assessed daily. At termination, spinal cords were assessed for cytokines, histopathology, and single cell RNA-sequencing. MRT-6160 significantly inhibited EAE disease progression, consistent with inhibition of T/B-cell stimulation-induced proliferation, differentiation, and pro-inflammatory cytokine production. *In vivo* activity was concomitant with reduced multiple sclerosis-relevant pro-inflammatory cytokines (IL-1β, IL-12p40, IP-10/CXCL10, MIP-1α, and IL-17A) and spinal cord immune cell infiltration, lesion area, and demyelination. Finally, single-cell transcriptomic analyses revealed that several key activation (TCR signaling, T cell checkpoint signaling, BCR signaling) and pro-inflammatory (Th17-mediated biology, Th1 and Th17 differentiation) pathways were reduced following treatment with MRT-6160. MRT-6160 attenuated both T- and B cell effector functions *in vitro* and demonstrated activity in a preclinical T/B-cell-mediated model of EAE. These data suggest that MRT-6160 may provide therapeutic benefit in patients with neuroinflammatory diseases among other T- and/or B-cell immune-mediated diseases.

W136. Proinflammatory Memory CD4$^+$ T Cell Markers Are Elevated in the Circulation of Patients with Juvenile Idiopathic Arthritis

Bella Lappalainen[1], Viola Pitkänen[1], Terhi Remes-Pakarinen[2], Paula Vähäsalo[3], Milja Möttönen[4], Minna-Maija Grönlund[4], Mikael Knip[5], Riitta Veijola[3], Jorma Toppari[4], Jorma Ilonen[4], Johanna Lempainen[5], Liisa Kröger[2], Anna-Mari Schroderus[1] and Tuure Kinnunen[1]

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Abstract Text: Juvenile idiopathic arthritis (JIA) is an autoimmune disease in children characterized by chronic inflammation of the joints. In patients with JIA, pro-inflammatory $CD4^+$ helper T cells have been suggested to play an important role in driving synovial inflammation. However, only limited data on peripheral blood immune cell changes exist in JIA patients. We studied circulating immune cells, in particular the memory $CD4^+$ T cell compartment, using multiparameter flow cytometry to compare patients with JIA (n=77) to healthy age- and sex-matched controls (n=53). Interestingly, we detected an elevated frequency of circulating $CD4^+$CXCR5-PD-1hi T peripheral (Tph) cells ($p< 0.0001$), a T cell subset suggested to provide help to B cells in inflamed tissues in patients with JIA compared to controls. Moreover, increased frequencies of tumor necrosis factor-alpha (TNF-α) ($p< 0.0001$), interleukin (IL)-2 ($p=0.0003$), and interferon-gamma (IFN-γ) ($p=0.0155$) producing $CD4^+$ memory T cells were detected in JIA patients compared to controls, when PBMCs were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin. In summary, the memory $CD4^+$ T cell compartment in patients with JIA shows alterations, characterized by T cell signatures linked to proinflammatory functions and enhanced T cell B cell interactions. These signatures could potentially be utilized in the development of T cell based biomarkers to monitor JIA disease activity.

W137. Peripheral Priming Is Required for Liver-Antigen-Specific TIGIT$^+$ PD-1$^+$ T Cell Accumulation in the Tissue

Thomas Guinebretière[1], Anaïs Cardon[2], Jean-Paul Judor[2], Pierre-Jean Gavlovsky[2], Richard Danger[2], Arnaud Nicot[2], Sophie Brouard[2], Jean-François Mosnier[3], Jérôme Gournay[4], Sophie Conchon[2] and Amédée Renand[2]

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Abstract Text: Purpose: In autoimmune hepatitis (AIH), the alteration of the hepatic tolerance is linked to an accumulation of autoreactive T cells. However, studying the emergence and biology of these T cells is technically challenging. The objective of this work was to decipher immune events involved in initiation and regulation of local hepatocellular-antigen-specific immune response. Methods: We developed a murine model, in which the model antigen hemagglutinin (HA) expression is inducible and restricted to hepatocytes, thanks to the Cre-Lox recombination system. Liver HA expression was induced through intravenous injection of Cre-coding adenovirus (Ad Cre) or tamoxifen treatment. Peripheral immunization was obtained by intramuscular injection of Ad Cre or HA-coding plasmid. HA-specific T cells were tracked and characterized through tetramer staining, flow cytometry and RNA-sequencing. Immuno-phenotyping of blood memory T cells from AIH patients was performed by spectral cytometry. Results: Local inflammatory signals are not sufficient to initiate adaptive immune response against HA when expressed in the liver. HA-specific T cell recruitment in the liver depends on peripheral priming combined with local antigen expression. Liver-antigen-specific T cell reactivity is marked by TIGIT and PD-1 co-expression. In AIH patients, the frequency of circulating PD-1$^+$TIGIT$^+$HLA-DR$^+$ T cells correlates with AIH disease activity. Conclusion: Our findings reveal a key connection between the liver and the periphery in hepatocellular-antigen-specific responses. TIGIT and PD-1 co-expression by T cells is a marker reflecting local T cell reactivity. Their presence in the blood of AIH patients suggests recirculation of autoreactive T cells and provides new biomarkers and therapeutic perspectives.

W138. Preclinical Polypharmacology of S-1117, a Novel Engineered Fc-Fused IgG Degrading Enzyme, for Chronic Treatment of Autoantibody-Mediated Diseases

Tobias Green, Liliana Sanmarco, Alex Pellerin, Jordan Anderson, Nathan Rollins, Agustin Plasencia, Andita Newton, Cecilia Ramello, Ryan Peckner, Yi Xing, Heather Vital, Nathan Higginson-Scott, John Sundy, Kevin Otipoby and Ivan Mascanfroni

Seismic Therapeutic, Watertown, MA

Abstract Text: Tobias Green, Liliana Sanmarco, Alex Pellerin, Jordan Anderson, Nathan Rollins, Agustin Plasencia, Andita Newton, Cecilia Ramello, Ryan Peckner, Yi Xing, Heather Vital, Nathan Higginson-Scott, John S. Sundy, Kevin L. Otipoby, Ivan Mascanfroni Seismic Therapeutic, Watertown, MA USA Pathogenic autoantibodies are key effectors of inflammation, promoting immune cell responses that cause tissue damage in autoantibody-mediated diseases. IgG-proteases represent a new therapeutic opportunity. Here we present S-1117, a novel Fc-fused pan-IgG protease, engineered using a proprietary machine learning enabled platform to reduce immunogenicity and augment manufacturability and stability while maintaining enzyme activity. S-1117 cleaves all IgG subclasses in human plasma. It directly eliminates IgG effector function, reducing antibody-dependent and complement-dependent cytotoxicity, antibody-dependent cellular phagocytosis, and IC-mediated immune cell activation *in vitro*. Moreover, S-1117 cleaves the IgG^+ BCR on human memory B cells *in vitro*. *In vivo*, a single dose of S-1117 provides greater (>90%), faster (< 24 hours), and more prolonged (>10 days) IgG reduction compared to a benchmark FcRn inhibitor (maximal IgG reduction of ~70% at 7 days). This correlates with superior efficacy in prophylactic and therapeutic murine models of ITP. Human PK/PD modeling predicts that infrequent chronic low doses of S-1117 can achieve a range of IgG reductions up to 90% or greater, titrated to the clinical needs of each patient. Advantages of enzymatic degradation, sustained PK and titratable PD demonstrate superiority in human IgG cleavage compared to a benchmark FcRn inhibitor and are expected to enable a convenient patient-tailored treatment regimen.

W139. Recurrent Immune Thrombocytopenia Unmasking an Atypical CVID Phenotype

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Abstract Text: Common Variable Immunodeficiency (CVID) is a primary immunodeficiency characterized by impaired B cell differentiation and defective antibody production, typically resulting in recurrent infections, autoimmune manifestations, and increased malignancy risk. However, significant clinical heterogeneity exists, and autoimmune cytopenias may be the predominant or initial presentation. We present the case of a Caucasian female diagnosed with immune thrombocytopenia (ITP) at age 18, achieving remission with corticosteroids. Her history included cold-induced urticaria, pernicious anemia, and iron deficiency anemia, but no significant infections. At age 21, she experienced an ITP relapse with severe thrombocytopenia (nadir: 2×10^9/L). Immunologic workup revealed selective IgA deficiency with normal IgG and IgM levels but impaired vaccine-specific responses. Flow cytometry showed preserved T cell counts but abnormal B cell maturation, including decreased class-switched memory B cells ($CD19^+CD27^+IgD^-$, 6%), reduced unswitched memory B cells ($CD19^+CD27^+IgD^+$, 1%), and elevated naive B cells ($CD19^+CD27^-IgD^+$, 83%). Genetic testing identified variants of uncertain significance (VUS) in FOXI3 and SAMD9, genes involved in immune and hematopoietic regulation, potentially contributing to the underlying immune dysregulation. Treatment with corticosteroids and IVIG led to sustained ITP remission. This case highlights the clinical diversity of CVID and supports the recognition of ITP as a potential dominant or sole manifestation. Recurrent autoimmune cytopenias, even in the absence of marked hypogammaglobulinemia or infections, should prompt evaluation for CVID. Early immunophenotyping and genetic analyses can clarify the diagnosis, enhance therapeutic planning, and improve clinical outcomes.

W141. Redirecting TCR Specificity in Regulatory T Cells Toward Class I HLA Antigens Mediates Tissue-specific Homing

Yannick Muller[1], Raphael Porret[1], Fanny Lebreton[2], Ana Alcaraz[1], Oscar Alfageme-Abello[1], Evangelos Stefanidis[3], aikaterini semilietof[4], Philippe Guillaume[7], Rebecca Cecchin[1], Georgakis Spyridon[1], Laura Ermellino[1], Erica Lana[1], Eleonora Pace[1], greta. Giordano[4], Vincent Zoete[1], Petrovas Konstantinos[1], Melita Irving[4], Ekaterine Berishvili[2] and Qizhi Tang[3]

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Abstract Text: Background Type 1 diabetes (T1D) is marked by the overexpression of class I major histocompatibility complex (MHC) antigens in pancreatic islets, which are targeted by islet-specific $CD8^+$ T cells. Here, we aimed to improve regulatory T cell (Treg) infiltration into pancreatic islets by redirecting their specificity toward class I-restricted islet antigens. Methods Two HLA-A2 (*02:01) restricted TCRs were generated, one specific for ZnT8186-194 (clone D222D), the second for IGRP265-273 (clone 32), and were inserted into the TRAC locus of primary T cells and Tregs by homology-directed repair. In parallel, the CD4 co-receptor was swapped with either CD8 or CD8 molecules into the CD4 locus. Results The functional avidity of clone D222D was peptide-specific, CD8β dependent, and mediated *in vivo* trafficking towards transgenic cell lines expressing the Znt8 peptide but not $HLA\text{-}A2^+$ human pancreatic islets. Interestingly, clone 32 exhibited antigen promiscuity despite a tetramer-specific staining, CD8α dependency, and mediated trafficking into HLA-A2 human pancreatic islets. Swapping the CD4 co-receptor to the CD8 and CD8 in engineered D222D and 32 CD4 T cells, respectively, enables specific recruitment *in vivo*. Engineered CD4to8 TCR Tregs maintained stable phenotypes, with significantly higher suppression activity than their polyclonal counterpart and showed co-receptor-dependent migration *in vivo*. Conclusion Tregs can be redirected against class I MHC-restricted islet antigens by co-engineering their TCRs and co-receptor. This approach demonstrates that TCR specificity, reflected by its functional activity, is crucial for tissue-specific trafficking, paving the way to improve the efficacy of Treg therapies for T1D.

W142. Sex Differences in the Human Gut Microbiota Contribute to CNS Autoimmunity

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Abstract Text: Women are at increased risk for autoimmune disease due to a variety of genetic and environmental risk factors. The human gut microbiota also differs by biological sex; however, the degree to which sex differences in the gut microbiota impact autoimmune disease remains largely unexplored. Herein, we leverage high-quality, multi-cohort metagenomic data to reveal a strong signature of biological sex in the human gut microbiota. Female-enriched bacterial species are enriched in multiple autoimmune diseases, including multiple sclerosis (MS). E. lenta exhibited the greatest female-enrichment, was enriched in MS patients, and exacerbated disease severity in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS-like neuroinflammation. E. lenta colonization significantly increased T-helper 1 (Th1) cells in the brain and T-helper 17 (Th17) cells in the gastrointestinal tract and brain in EAE, elevating interferon-γ (IFNγ) and interleukin-17a (IL-17a), the major cytokines that drive EAE. Heat-killed E. lenta cells induced IFNγ in primary $CD4^+$ T cells from healthy mice and E. lenta monocolonization induced expansion of $CD4^+IFN\gamma^+$ T cells in the gut of healthy animals, suggesting that E. lenta induces classic Th1 inflammatory cytokine IFNγ independent of diseases state. These data add to the growing literature implicating E. lenta in the risk of autoimmunity and other diseases and further our understanding of the unique influence E. lenta exerts on host immune response. Moreover, these data underscore the importance of

biological sex in shaping host-microbiome interactions as well as our need to better understand the microbial etiology of sex bias in autoimmunity.

W143. Single-cell Chromatin Accessibility Identifies Dysregulated Transcription Factor-gene Networks in Peripheral Blood of Lupus Patients

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AbbVie, Malden, MA

Abstract Text: Autoimmune disorders such as systemic lupus erythematosus (SLE), result from a complex interaction of genetic and environmental factors. Understanding cell type-specific epigenetic processes and how they interact with genetics remains a critical yet underexplored area. In the current project, we generated the largest single-cell chromatin accessibility profile of human peripheral blood mononuclear cells (PBMC) of any autoimmune disease to date to interrogate genetic-epigenetic interactions in cell type-specific manner. We generated over 1.1 million single-cell chromatin accessibility (scATACseq) profiles using PBMCs from a cohort of 70 SLE patients and healthy controls from two ethnic groups. We identified subsets of Bcell memory, and $CD8^+$ effector memory T cells were expanded in SLE. We performed differential peak accessibility analysis within each major PBMC cell type and identified 9,104 differentially accessible peaks across PBMC cell types in known and novel genes including cytokine signaling and receptors such as CXCR4 and IL7R, integrin family such as ITGB2, ITGAM (CD11b), CORO1A. Transcription factor motif enrichment analysis defines key transcription factor regulators in SLE across PBMC cell types including AP-1 factors FOS, JUN, BACH in lymphocytes and CEBP family in monocytes modulating critical gene networks. Incorporation of genotyping enabled Chromatin accessibility quantitative trait loci (caQTL) analysis identifies causal SLE GWAS risk variants regulating accessibility at key genes including SLC15A4 and OVOL1. This research provides novel insights into the genetic and epigenetic transcription factor gene regulation of SLE in pathogenic immune cell types. These findings offer a new depth of understanding in SLE pathogenesis and potential therapeutic targets.

W144. Stem-like Peripheral Helper T Cells Seed Their Effector Counterpart in Rheumatoid Arthritis

Yuki Masuo[1], Akinori Murakami[1], Rinko Akamine[1], Osamu Iri[1], Shunsuke Uno[1], Koichi Murata[1], Kohei Nishitani[1], Hiromu Ito[1], Ryu Watanabe[2], Takayuki Fujii[1], Takeshi Iwasaki[1], Shinichiro Nakamura[1], Shinichi Kuriyama[1], Yugo Morita[1], Yasuhiro Murakawa[1], Chikashi Terao[3], Yukinori Okada[4], Motomu Hashimoto[2], Shuichi Matsuda[1], Hideki Ueno[1] and Hiroyuki Yoshitomi[1]

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Abstract Text: Peripheral helper T (Tph) cells play important pathogenic roles in human autoimmune diseases. Tph cells are proposed to be the major B cell helpers in inflamed joints of rheumatoid arthritis (RA). However, whether and how Tph cells are engaged in tissue inflammation remains unclear. Here, we demonstrate that Tph cells consist of two directly related subsets in RA: stem-like Tph (S-Tph) and effector Tph (E-Tph) cells. These two subsets differed in transcriptome, epigenome, B-helper capacity, spatial localization, and the encountering cells. S-Tph cells were endowed with a self-renewal capacity, which was dependent on TCF1. S-Tph cells were mainly found at the center of tertiary lymphoid structures (TLSs) and colocalized with B cells. S-Tph cells potently induced B cells to produce immunoglobulins. S-Tph cells expressed CCR7 and were in proximity to $CCL19^+$ perivascular fibroblast-like synoviocytes in TLSs. By contrast, E-Tph cells highly expressed effector molecules including IFN-g. E-Tph cells expressed CXCR6, CCR2, and CCR5, and $CXCL16^+$ proinflammatory macrophages and $CCL4^+$ $CCL5^+$ $CD8^+$ T cells likely promoted E-Tph cells migrating outside TLSs. S-Tph and E-Tph cells robustly shared TCR clonotypes, and S-

Tph cells were able to differentiate into E-Tph cells upon TCR stimulation and coculture with B cells, but not the opposite. Collectively, our study shows that S-Tph cells play a central role in promoting Tph responses by undergoing self-renewal and seeding E-Tph cells. Our study provides a rationale to target S-Tph cells for the treatment of human autoimmune diseases with an expectation to reduce global Tph responses and TLS formation.

W145. Targeting IRF5: Discovery and Preclinical Development of Selective Small Molecule Inhibitors

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Abstract Text: Interferon regulatory factor 5 (IRF5) is a transcription factor that regulates inflammatory cytokine and type I interferon production downstream of Toll-like receptors (TLRs) 7, 8, and 9. Genetic polymorphisms in IRF5 are associated with increased risk for systemic lupus erythematosus (SLE), Sjögren's syndrome, and other autoimmune diseases, underscoring its potential as a therapeutic target. IRF5-deficient mice exhibited strong disease protection in several models of SLE, with reductions observed in type I interferon levels and anti-nuclear autoantibodies. Importantly, heterozygous IRF5 mice also showed substantial protection from disease development, suggesting that target modulation, but not complete inhibition, may be sufficient for therapeutic benefit. Despite the challenges of targeting a transcription factor lacking a conventional active site, we have successfully discovered potent and selective small molecule IRF5 inhibitors. These novel compounds effectively blocked IRF5 phosphorylation, nuclear translocation, and subsequent cytokine production in human monocytes following TLR7/8 stimulation with >100-fold selectivity over IRF3, IRF7, and the adjacent TLR2 pathway. *In vivo*, oral delivery of the IRF5 inhibitor downregulated type I interferon signature genes in a short-term pristane-induced lupus model and significantly reduced joint inflammation and autoantibody production in both methylated bovine serum albumin (mBSA) and collagen-induced arthritis (CIA) models. These compelling preclinical data support IRF5 inhibition as a promising therapeutic strategy for autoimmune diseases and the continued advancement of our IRF5 inhibitor program.

W146. Therapeutic development for Axial Spondyloarthritis: ERAP-1 inhibition by GRWD0715 results in auto-antigen loss on HLA-B*27 and the inhibition of cognate CD8$^+$ T cell responses.

Samantha Bucktrout[1], Kris Clark[1], Daniel Green[1], Martin Quibell[1], Cheryl McAlpine[1], Bethany Kuszlewicz[1], Jason Shiers[2], Emma Teal[3], Helen Tunbridge[3], Anamarija Pfeiffer[4], Daniel Redfern[4], Tsang Wai Lam[5], Mohammed Hassan[6], Lama Kalouche-Khalil[1], Tom Lillie[1], Peter Joyce[1] and Laura Quinn[1]

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Abstract Text: HLA-B27$^+$ axial spondyloarthritis (axSpA) is a progressive autoimmune disease with a prevalence ranging from 0.1 to 1.4%. It is managed with anti-inflammatory agents, but fewer than 50% patients achieve remission that is rarely curative, leaving a high unmet need. The association of endoplasmic reticulum amino peptidase 1 (ERAP1) with axSpA is strong ($p < 10\text{-}50$) increasing to $p=6x10\text{-}54$ in epistasis with HLA-B*27. ERAP1 activity determines which antigenic peptides are available for adaptive immunity, trimming longer peptides to ~9 amino acids (a.a). An hypomorphic allele of ERAP1 is highly protective for HLA-B*27$^+$ axSpA, supporting ERAP1 inhibition in axSpA. It's hypothesised ERAP1 inhibition will prevent auto-antigen generation and pathogenic CD8$^+$ T cell activation leading to disease resolution. Our highly potent and selective ERAP1 inhibitor GRWD0715 will start testing in the second half of 2025. Here we demonstrate ERAP1 cleavage of putative axSpA auto-antigen precursors derived from GLRB, HelQ and SEC14L2, with GRWD0715 inhibiting the formation of auto-antigens at an IC50 ranging from 60-400 pM. 7-9 a.a. peptides were significantly down regulated on HLA-B*27 molecules expressed on

joint-relevant cell lines exposed to GRWD0715. GRWD0715 suppressed the activation of axSpA-associated-TCR^{+} CD8^{+} T cells in an HLA-B*27-autopeptide-dependent manner. We present in silico analysis that implicates GRWD0715-mediated loss of auto-antigen-HLA-B*27 resulted from the derivation of incompatible a.a. residues at the P2 anchor position. Together these data support the development of GRWD0715 as a disease-modifying therapy for axSpA and other HLA-B*27-associated autoimmune disease through the removal of HLA-I-presented autoantigens and the inhibition of auto-reactive CD8^{+} T cell responses.

W147. Unveiling the Role of IL-12A in Lupus: Linking Genetic Risk to B Cell Pathogenicity

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Abstract Text: Systemic lupus erythematosus (SLE) is a complex autoimmune disease with over 200 risk loci identified through genome-wide association studies (GWAS). Among these, a risk locus harbouring the gene IL12A remains understudied. IL12A participates in the activation of the IFN-γ pathway. IFN-γ signalling drives B cell differentiation into IgD- CD27^{-} CD21^{-} CD11c^{+} (DN2) B cells, a likely pathogenic subset expanded in SLE patients. We hypothesize that an SLE risk variant dysregulates IL12A, which impacts DN2 B cell differentiation. First, we narrowed down the candidate causal risk variants by their overlap with regulatory elements in a B cell line. We identified a single nucleotide polymorphism (SNP) within a strong enhancer region near IL12A. Using CRISPR inactivation, we showed that silencing this enhancer significantly reduces IL12A expression. Additionally, we used base editing to show that the risk allele of this SNP increases IL12A expression. Notably, this SNP overlaps an open chromatin region (ATAC-seq) in primary B cells activated *in vitro* with a DN2-inducing cocktail. To investigate IL-12A's functional role, we performed CRISPR knockout of IL-12A in primary naive B cells, which caused a decrease in DN2 population differentiation compared to the control. Conversely, treatment with recombinant IL-12A increased the DN2 population. Overall, our findings identify a likely causal SNP at the SLE IL12A locus, with the risk allele driving increased IL12A expression in a B cell line. Moreover, our data showed that IL12A plays a direct role in promoting DN2 B cell differentiation, linking this locus to pathogenic mechanisms in SLE.

Autoinflammatory Diseases

Th124. Long-term Viral Presence in Monocytes Correlates with Dysregulation of Innate Immunity in Patients with Multisystem Inflammatory Syndrome in Children (MIS-C)

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Abstract Text: MIS-C is a severe post-infectious complication of SARS-CoV-2, with a known pathogenic role of monocytes, yet mechanisms sustaining inflammation remain unclear. Genetic variants in the OAS/RNase L pathway, involved in viral double-stranded RNA (dsRNA) sensing and inflammatory modulation, was linked to MIS-C. We analyzed PBMCs, plasma, and RNA from MIS-C patients, using a multifaceted approach to study NLRP3 inflammasome activation, blood biomarkers, autoantibody profiling, transcriptomics, and proteomics. PBMCs, plasma, and RNA samples from MIS-C patients were analyzed. PBMCs were stimulated with LPS and ATP, and inflammatory responses were quantified using ELISA, flow cytometry, and imaging flow xytometry. Type-I interferon signature was measured using qPCR. RNA-sequencing data were analyzed through bioinformatics pipelines, including gene set enrichment analysis, while proteomics data were processed using weighted gene co-expression network analysis. We found a monocyte exhaustion phenotype, demonstrated by reduced inflammasome activation in response to stimuli and an associated lack of IL-1β secretion. Intriguingly, dsRNA was detected in monocytes from MIS-C patients even during follow-up, suggesting a long-lasting viral presence that could drive sustained immune activation. Blood biomarker profiling, bulk RNA-seq, and proteomic analyses revealed a signature characterized by the activation of genes involved in antiviral response, systemic inflammation, oxidative stress, and coagulation pathways. Additionally, we observed anti-IL1RA autoantibodies in 40-50% of patients, consistent with previous reports. Our findings highlight immune dysregulation in MIS-C, characterized by monocyte exhaustion, intramonocytic dsRNA persistence, autoantibodies, and distinct transcriptomic/proteomic signatures. These insights provide a deeper understanding of mechanisms underpinning sustained inflammation in post-COVID syndromes.

Th125. Neutrophil Inflammation and Platelet Activation Underlie Organ Failure in Acute Pancreatitis

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Abstract Text: Acute Pancreatitis (AP) is an enigmatic disorder caused by autodigestion of the pancreas, which can either result in a mild self-limiting form or can progress to a severe form characterized by distant organ failure which carries a high rate of mortality. There is relatively limited data describing the immunology of acute pancreatitis, and thus, significant interest given that this is a highly inflammatory condition. In this study we employed longitudinal whole blood RNA sequencing and matched complete blood count panels following hospitalization to determine the molecular signatures which differentiate mild versus severe AP. Participants with more severe AP demonstrated elevated transcriptional signatures of neutrophil-driven inflammation and platelet activation which mirrors clinical findings. In contrast, participants with mild AP demonstrated higher expression of B and T lymphocyte genes, indicating an innate versus adaptive immune axis in the progression or resolution of AP. While most immune-related processes return to baseline quickly, a subset of megakaryocyte genes remain elevated in severe cases revealing a new potential route of interrogation to understand severe complications post-AP. Finally, by employing complementary machine learning-based approaches we confirm that transcriptional networks enriched for neutrophil-mediated inflammation are a hallmark of more severe disease. Together, our data indicates that neutrophil driven inflammation and coagulopathy may underlie the spread of damage from the pancreas to other organs, which presents a potential opportunity for better predicting patient risk and devising new treatment options.

Th126. Phenotyping of Human UC Colonic Tissue Reveals Inflammatory Pathway Gene Expression in PD-1$^+$ Conventional and Regulatory T Cells Which Overlap with Those Regulated by Rosnilimab in a Mouse Model of Colitis

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Abstract Text: In ulcerative colitis (UC), the lamina propria has multiple T cell subtypes that express the co-inhibitory receptor, PD-1, including conventional T effector memory cells and T peripheral helper cells (Tph). The reduction of PD-1$^+$ Tph cells is correlated with remission, therefore PD-1 receptor agonists, such as rosnilimab, are a promising class of therapeutics that leverage immune homeostatic mechanisms to regulate T cell activity for treatment of inflammatory diseases, including UC. PD-1$^+$ T cell subsets were characterized via secondary analyses of a single-cell data set from UC patient-derived colonic tissue and showed PD-1$^+$ CD4 conventional T cells and PD-1$^+$ Tregs had increased JAK-STAT, TNF, and NF-κB estimated pathway activity compared to PD-1$^-$ cells. PD-1$^+$ Tregs had increased expression of genes associated with a pro-inflammatory phenotype, including TNF, TBX21(TBET), and IL12RB2, consistent with the hypothesis that PD-1$^+$ Tregs may be a pro-inflammatory subtype. In a preclinical study, genes in pathways involved in human UC ("Signaling by interleukins" (e.g., Il6) and "Chemokine receptors bind chemokines" (e.g., Ccl20)) were upregulated in mouse colitis, compared to naive animals. These genes, in addition to the barrier gene Muc3 and fibrosis-associated Mmp9, were normal in rosnilimab-treated mice while dysregulated in colitis, and corresponds to published data showing rosnilimab reduced body weight loss, reduced inflammation of the colon, and reduced infiltration of CD4 T cells in mouse colitis. These analyses suggest that PD-1 is upstream of multiple clinically validated drivers of UC, supporting the scientific rationale for studying rosnilimab in an ongoing Phase 2 study in UC (NCT06127043).

Th127. Proteomic Signatures Distinguish Common Variable Immunodeficiency with Immune Dysregulation from Related Immune-mediated Inflammatory Diseases

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Abstract Text: Introduction Common Variable Immunodeficiency with immune dysregulation (CVIDid) is the primary cause of morbidity and mortality in CVID patients. Clinically, CVIDid may resemble other immune-mediated inflammatory diseases (IMIDs) like Sjögren's Syndrome and rheumatological conditions. Through comparing serum proteomic profiles of CVID and related IMIDs we aimed to clarify whether CVIDid should be regarded as a dinstinct disease entity. Methods We analyzed 315 serum samples, quantifying 92 immune-related proteins using a proximity extension assay in healthy controls and patients with Atopic Dermatitis, Sjögren's Syndrome, Checkpoint Inhibition toxicity, Rheumatoid Arthritis, CVIDid, and CVID with infections only (CVIDio). Feature selection was performed via elastic net regression, followed by cluster analysis to identify distinct proteomic patterns. Results CVIDid exhibited a unique T/NK-cell cluster featuring CRTAM, KLRD1, CD8A, but also IL10, suggesting heightened cytotoxic activity and chronic inflammation. Atopic Dermatitis showed a prominent Th2-skewed profile (MCP4, CCL17), whereas Rheumatoid Arthritis and Sjögren's Syndrome showed more pronounced proinflammatory (IL-6, TNF) and IFN-driven (CXCL11, Galectin-9) signatures, respectively. Checkpoint Inhibition toxicity showed exceptionally high PDCD1 and IFN-γ. Notably, lymphoid organ related proteins (CXCL13, CCL19, ICOSLG, LAMP3) were more pronounced in CVIDid, underscoring a dysregulated yet active lymphoid microenvironment. CVIDio showed a dampened inflammatory signature, emphasizing distinct immunopathology from CVIDid. Conclusions Proteomic profiles confirm CVIDid as a distinct disease entity, characterized by high T/NK-cell activity and an active lymphoid microenvironment, despite poor antibody production. This paradox points to a chronic inflammatory state where multi-targeted therapies, such as JAK-inhibitors, may be effective treatment options.

Th128. TIM-3 Interactome Finetunes Regulatory Activity of Innate Immunity in Autoimmune Diseases

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Abstract Text: Immune co-inhibitory receptors are essential for maintaining immune cell homeostasis. TIM-3, initially identified on terminally differentiated T cells, is now recognized as being expressed on various immune cell types, including myeloid cells. However, its regulatory mechanisms in myeloid cells, particularly in autoimmune diseases, remain poorly understood. Germline loss-of-function mutations in TIM-3 have been linked to subcutaneous panniculitis-like T cell lymphoma (SPTCL), often associated with autoimmune disorders. Reduced TIM-3 surface expression on myeloid cells in multiple sclerosis (MS) patients further underscores its role in immune regulation. Using CRISPR-Cas9 to knock out TIM-3 in human primary myeloid cells, we observed increased inflammasome activation and elevated chemokine secretion (CXCL9, CXCL10), promoting a pro-inflammatory microenvironment. Phosphoproteome profiling identified key mediators of TIM-3 signaling, including LYN, HSP60, NFκB, and MAP kinase pathways. Co-immunoprecipitation and mass spectrometry revealed MHC-class 1, CD22 and LYN as one of TIM-3 interaction partners. Interestingly, CD22, a co-inhibitory receptor typically found on B cells, was also detected on microglia in the human brain, where it appears to function as an inhibitory regulator by modulating immune responses. These findings highlight the TIM-3-CD22-LYN axis as a novel pathway in immune regulation and suggest it as a promising therapeutic target for autoimmune diseases like SPTCL and MS.

Tu138. A Rat Model of Chronic Colitis with Fibrosis Induced by Repeated Intrarectal TNBS Administration

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Abstract Text: Intestinal fibrosis is a frequent complication associated with chronic inflammation in patients with Inflammatory Bowel Diseases (IBD) such as Crohn's Disease (CD). While therapies exist for inflammation related to IBD, there are no approved therapeutics for intestinal fibrosis. Additionally, most animal models for IBD do not effectively model the chronic transmural inflammation or intestinal fibrosis observed in CD. With the goal of establishing a rodent model to test novel therapeutics for intestinal fibrosis, we induced colitis/inflammation with weekly intrarectal TNBS administration and tracked the progression of colitis and fibrosis. TNBS-administered animals demonstrated significantly reduced body weight gain and significant increases in Disease Activity Index scores compared to naive rats. Additionally, significant levels of colitis were observed by video endoscopy following TNBS, and terminal measures of colon weight:length ratios were significantly increased in diseased animals on Day 39. Colon samples were processed for histopathology analysis, showing significant increases in inflammation, necrosis/gland loss, and epithelial hyperplasia in TNBS-dosed animals. Importantly, erosions and ulcerations were successfully induced, with significantly increased mural (submucosal and tunica muscularis) inflammation. Granulation tissue and collagen I/III protein (IHC) deposition were associated with erosions and ulcerations; submucosal collagen I/III immunolabeling normalized to colon length was significantly increased in diseased animals. Collectively, these data show that repeated TNBS administration provides a validated approach to induce clinically-relevant features of intestinal mural fibrosis associated with chronic inflammation, allowing for assessment of novel therapeutics for this clinically unmet need.

Tu139. Deficiency of Human Adenosine Deaminase 2 (DADA2)- the Oxford Experience

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Abstract Text: Deficiency of adenosine deaminase type 2 (DADA2) is a rare inherited autoinflammatory condition, first described in 2014, with a prevalence of ~1 in 222,000. It is characterized by systemic vasculitis, stroke, cytopenia, and immunodeficiency. We report three cases from Oxford *Clinical Immunology*. Two siblings presented with systemic vasculitis and strokes. The first experienced acute ischaemic infarcts at 11 and 18 years, with no identifiable cause in routine investigations. Whole genome sequencing revealed compound-heterozygous ADA2 mutations: c139G>A (maternal) and c100C>T (paternal). She was initially managed with Aspirin and Inclisiran. Her sister, presenting at 12 years with diplopia and upward gaze nystagmus, was diagnosed with infarcts in the pons and left internal capsule and confirmed to have the same mutations. Immune phenotyping was normal in both, and they are managed with Etanercept for secondary prevention. A third patient presented at 5 weeks with progressive neutropenia, febrile episodes, recurrent infections, failure to thrive, and microcephaly. Despite normal routine investigations, whole genome sequencing identified homozygous ADA2 exon 7 deletion. Bone marrow aspiration revealed impaired myelopoiesis and maturation. She underwent a bone marrow transplant at 1.5 years with a complex recovery. These cases highlight the diverse presentations of DADA2 and the diagnostic challenges it poses. A multidisciplinary approach is essential for timely diagnosis and effective management of this rare condition.

Tu140. FAM83H Is Involved in Normal Lymphopoiesis in the Postnatal Hematopoietic Niche

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Abstract Text: FAM83H (family with sequence similarity 83 member H) is mostly expressed in epithelial cells and is associated with intracellular transport, cytoskeletal regulation, and enamel formation. Mutations in Fam83h cause amelogenesis imperfecta (AI), characterized by defective enamel. We generated Fam83h knockout (KO) mice using CRISPR/Cas9. While enamel defects were absent, KO mice exhibited reduced body size, scruffy coats, and scaly skin. They developed inflamed front paw lesions, likely caused by a disrupted skin barrier. Elevated neutrophil levels in peripheral blood and impaired leukocyte development, including T, B, and NK cells, were observed in KO mice; however, the soft tissue lesions resolved and returned to normal by 7 weeks of age. Specifically, at two weeks, the thymi of KO mice have reduced capacity to generate T cells, with a partial arrest at the DN stage, and thymic architecture is disturbed. Our findings show that FAM83H primarily expressed by stromal cells, plays a crucial role in modulating lymphoid cell development within postnatal hematopoietic niches. Furthermore, based on our expression data, FAM83H attenuates Wnt signaling by promoting CK1-dependent degradation of β-catenin in postnatal thymic epithelial cells (TECs). These findings highlight FAM83H's vital role in hematopoietic niches and immune cell development.

Tu141. HSID: An Integrative Target Discovery Framework for Hidradenitis Suppurativa

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Sanofi

Abstract Text: Hidradenitis suppurativa (HS) is a chronic skin condition affecting intertriginous skin areas with apocrine sweat glands. It's characterized by painful, pus-filled bumps that can develop into tunnels beneath the skin. HS causes significant physical and emotional distress due to its recurrent nature, infections, and potential scarring. Current treatments, including TNF and IL-17 blockers, have limited success in resolving the disease, with many patients experiencing flares despite ongoing therapy. Surgical interventions, while sometimes necessary, carry risks and may not fully resolve the condition. These challenges highlight the urgent need for new therapeutic approaches

to address HS. To address this, we developed a comprehensive HS target engine which integrates human genetics data from four GWAS studies totaling over 3100 cases and 940k controls and curated disease mutations from HGMD, 16 bulk and single-cell transcriptomics studies from internal and public datasets, network biology, natural language processing extracted literature data, a knowledge graph, and genome-wide target draggability data. Using this framework, we rank and prioritize every gene by scoring them based on the strength and consistency of the integrated evidence. The top-ranked genes generated by the HS target engine successfully recovers known genes (IL1B, TNF, etc) implicated in HS biology (inflammation, tissue remodeling and destruction, stress responses, etc.) and identifies potential novel targets involved in skin immunity. Notably, clinically validated targets are enriched, with three of the top 20 targets corresponding to approved drugs for HS. This highlights the strength of our framework in uncovering both validated and novel HS therapeutic opportunities.

Tu142. Investigating Autoantibody Specificities in Lupus Target Organs: A Correlation with Inflammatory Biomarkers

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Abstract Text: Systemic lupus erythematosus (SLE) is characterized by dysregulated immune responses, often leading to organ damage. B cells play a key role in lupus pathogenesis, as evidenced by the success of B cell targeting therapies. While serum autoantibody profiles are well-studied and can serve as biomarkers of lupus, less is known about B cell reactivity in target organs. To address this gap, we analyzed autoantibody profiles in cerebrospinal fluid (CSF) of neuropsychiatric lupus (NPSLE) patients and interstitial skin fluid of cutaneous lupus erythematosus (CLE) patients using a human immunoglobulin isotyping panel. Our findings indicate that the CSF of NPSLE patients contains IgG1, IgG3, and IgA. We also compared the blister fluid of CLE patients and healthy patients, and found that CLE patients demonstrate higher levels of IgM in their blister fluid, but lower levels of IgG3, IgG4, and IgA as compared to healthy controls. Finally, we analyzed the cytokine expression in these fluids to identify potential factors for B cell isotype class switching. NPSLE patients had higher levels of CXCL10 and IL-8 and lower levels of IL-1β and IFN-β as compared to other neuroinflammatory conditions. CLE patients had higher levels of multiple cytokines and chemokines, most notably IFN and IFN-inducible chemokines including CXCL10. We plan to analyze which autoantigens are recognized by IgM in the skin and IgG1/3 and IgA in the CSF. This study provides novel insights into organ-specific B cell reactivity in lupus, with implications for targeted therapeutic strategies.

W148. ADA2 Orchestrates Interferon Responses and Safeguards Hematopoietic Stem Cell Integrity

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Abstract Text: Adenosine deaminase 2 (ADA2) is an extracellular enzyme that catalyzes the conversion of adenosine to inosine. It is primarily expressed and secreted by myeloid cells, particularly monocytes and macrophages, and plays a critical role in modulating inflammatory immune responses. Loss-of-function mutations in the ADA2 gene cause Deficiency of Adenosine Deaminase 2 (DADA2), a systemic autoinflammatory disorder characterized by vasculitis, immunodeficiency, and bone marrow (BM) failure. A hallmark of DADA2 is a pronounced type I interferon (IFN) signature, which contributes to disease pathogenesis; however, the precise role of ADA2 in IFN signaling and hematopoietic dysfunction remains unclear. We identified the cGAS–STING and JAK–STAT pathways as key mediators of the exaggerated IFN response in DADA2, supporting the rationale for targeted pharmacological inhibition. Furthermore, restoration of ADA2 function through gene therapy normalized IFN signaling, highlighting its potential as a long-term curative approach. In DADA2 patients, hematopoietic stem and

progenitor cells (HSPCs) display reduced frequency and clonogenic potential, even in the absence of overt hematological symptoms. Single-cell RNA sequencing revealed widespread transcriptional alterations across all HSPC subtypes. Additional studies showed that DADA2 mesenchymal stromal cells are functionally impaired and contribute to hematopoietic defects, pointing to both cell-intrinsic and niche-driven mechanisms of bone marrow failure. Together, these findings define ADA2 as a critical regulator of inflammatory balance and hematopoietic homeostasis, advancing our understanding of DADA2 pathogenesis and uncovering broader principles of bone marrow failure.

W149. EVO756: A Promising Oral MRGPRX2 Inhibitor Showing Strong Preclinical and Phase 1 Results

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Abstract Text: Mas-Related G-Protein Coupled Receptor X2 (MRGPRX2) is a versatile receptor predominantly found on mast cells and peripheral sensory neurons. It is a key player in FcεRI-independent mast cell degranulation and drives neurogenic inflammation linked to symptoms such as cough, pain, and itch. MRGPRX2's activation by a wide array of ligands present during chronic inflammation underscores its significant role in tissue pathophysiology. Consequently, targeting MRGPRX2 offers a promising therapeutic strategy for chronic inflammatory diseases like chronic spontaneous urticaria, atopic dermatitis or asthma. We introduce EVO756, a novel, oral small molecule inhibitor of MRGPRX2 with robust preclinical and clinical data. EVO756 effectively inhibits MRGPRX2-mediated responses in various models, including transfected CHO cells, LAD2 cells, human skin primary mast cells, and *ex vivo* skin explants. In a Phase 1, randomized, double-blind, placebo-controlled study, EVO756 demonstrated a well-tolerated safety profile and a pharmacokinetic profile that was proportional to the administered dose. Its pharmacodynamic activity was evaluated through a skin challenge test using icatibant, a known MRGPRX2 ligand. Treatment with EVO756 resulted in statistically significant reductions in icatibant-induced wheal sizes compared to placebo, confirming strong target engagement. These findings establish EVO756 as a well-tolerated, orally bioavailable inhibitor with the potential to modulate MRGPRX2-driven activation effectively. With promising results from preclinical and Phase 1 studies, EVO756 holds great potential as a treatment for diseases involving mast cells and neuro-inflammation. Further exploration is warranted to unlock its full clinical potential.

W150. Human ELF4 Deficiency Illuminates Its Role in Inflammasome Biology

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Abstract Text: We recently discovered a novel human inborn error of immunity (IEI) caused by loss-of-function variants in the X-linked ELF4 transcription factor. Pediatric male patients with Deficiency in ELF4, X-linked (DEX) suffer from recurrent fevers, oral ulcers, and mucosal-inflammation. Elevated CXCL1, IL-6, IL-18, and IL-12/IL-23, as well as clinical responsiveness to TNFalpha, IL-1, or IL-12p40 blockade, have been observed in DEX patients. To better understand the function of ELF4, we have generated mouse models of DEX. ELF4-deficient mouse macrophages exhibit a hyperinflammatory phenotype—yet the molecular drivers remain unclear. To address this question, we performed RNA-, ATAC-, and Cut-and-Run sequencing analysis of LPS-stimulated ELF4-deficient macrophages and wild-type (WT) macrophages. Here, we observed robust changes in the transcriptome, chromatin-accessibility, and epigenetic state of macrophages—including but not limited to effects on known pro-inflammatory genes. Specifically, in ELF4-deficient macrophages compared to WT-macrophages we find: (a) reduced chromatin-

accessibility, (b) diminished H3K4me3 and K3K27ac occupancy, (c) reciprocal regulation between Ifnb1 (attenuation) and IL-1/IL-18 (activation), and (d) a substantial enrichment in the Nod-like receptor (NLR) pathway. *In vitro*, both ELF4-deficient macrophages and neutrophils exhibited elevated inflammasome cytokine maturation in response to ATP/Nigericin but not flagellin, poly[dA-dT], or Val-boroPro at the concentrations tested. Notably, exogenous IFN-beta1 addition to ELF4-deficient cells attenuated inflammasome activity to WT levels. Thus, we have discovered a key role for the ELF4 transcription factor in regulating the magnitude of inflammasome activation in myeloid cells, providing insights into basic biology and offering potential therapeutic targets for inflammatory diseases.

W151. Identifying Distinct Subsets of Vγ5^{+} Epidermal Gamma Delta T Cells in Psoriasis Using Single-cell RNA Sequencing

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Abstract Text: The prevalence of autoimmune skin disorders is increasing in the U.S., with 3.0% suffering from psoriasis in 2024. Human skin contains resident T cells, including epidermal gd and ab T cells and dermal gd and ab T cells. Dermal gd T cells are well known IL-17 producers that contribute to psoriasis however, less is known about the epidermal subset. Using murine models of IMQ-induced psoriasis, we examined the role of distinct subsets of gd T cells in the epidermis. We analyzed publicly available scRNA-seq data to assess whether epidermal γδ T cell populations have distinct roles in psoriasis. We identified five distinct epidermal γδ T cell clusters in murine control and imiquimod-treated skin samples. The genes that define each cluster represent functional groups as such: cluster 1 Th1 responses (CD27, CD96, and IL12RB2), cluster 2 neuronal communication (INSR, NRCAM, and NPY1R), cluster 3 T regulatory function (KDM5D, Kat5, CD24a, and Itgae) cluster 4 T cell proliferation (Dcaf6, CD7, CD94, and CD247), and cluster 5 Th17 responses (IL17F, Rora). We are validating each epidermal γδ T cell cluster *in vivo* in an IMQ-induced psoriasis mouse model of disease using immunofluorescence and flow cytometry, Understanding the functions of epidermal γδ T cell clusters in psoriasis may reveal novel ways to control psoriasis.

W152. Increased Skeletal Muscle Autophagy Protects from Neutrophil-mediated Gut Inflammation

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Abstract Text: Inflammatory bowel disease (IBD), comprising of Crohn's Disease and Ulcerative Colitis, is a debilitating disease with no known cause. Although extensive work has linked numerous genetic loci to IBD, most of these associations remain incompletely understood. Here, we show that DUPD1/DUSP29, a dual-specificity phosphatase of unknown function, is exclusively expressed in the skeletal muscle, and negatively regulates autophagy in this tissue. As autophagy is a known risk factor in IBD, we tested the role of DUPD1 in colitis. Dupd1$^{-/-}$ mice were protected from dextran sodium sulfate (DSS) and Helicobacter hepaticus induced colitis in Il-10$^{-/-}$ mice, as well as DSS/Azoxymethane (AOM) induced colitis-associated colon cancer (CAC). Deletion of DUPD1 in the skeletal muscle (Dupd1fl/flMyf6Cre) protects mice from colitis. The increased autophagic flux in Dupd1$^{-/-}$ mice was associated with reduced neutrophil-mediated systemic inflammation and reduced neutrophil extracellular traps (NETs) formation in the colonic mucosae, a process known to impair intestinal barrier function and cause mucosal damage. Oral pharmacologic inhibition of DUPD1 catalytic activity led to increased autophagy flux in the skeletal muscle, and reduced colitis in wild type mice. This work shows that the skeletal muscle acts as an immunological organ capable of regulating inflammation at distant sites, and implicates DUPD1 as a potential novel therapeutic target in IBD.

W153. Local Delivery of IL-35 mRNA Therapeutic Using Lipid Based Nanoparticles Demonstrates High Preclinical Efficacy to Suppress Acute and Chronic Autoimmune Hepatitis

BELARIF LYSSIA, Damien Habrant, Emmanuelle Wilhelm, Sabrina Pengam, Marion Drouin, Clémence Gaudin, Virginie Aillerie, Amandine Georges, Julien Taurelle, Ariane Desselle, Caroline Mary, Aurore Morello and Nicolas Poirier

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Abstract Text: IL-35 is an immunosuppressive cytokine inhibiting multiple immune cells. Cytokine clinical use is limited by their short half-live and unwanted toxicity. To overcome these challenges and enable a local delivery at the inflammation site, we have developed an IL35-encoded mRNA therapy vectorized with Lipid-based-nanoparticles (LNP). IL-35 shows promising therapeutic potential for inflammatory diseases due to its pleiotropic immunosuppressive effect as we and others demonstrated by inhibiting activation of T/B, myeloid and fibroblast cells. In addition, IL-35 induces a long-term immunosuppressive loop effect by promoting regulatory B/T cells. Efficacy of our mRNA IL35/LNP strategy was evaluated in acute and chronic hepatitis mouse models. Acute Concavalin A model is characterized by acute inflammation and hepatotoxicity. IL35 mRNA treatment reverses periphery inflammation (pro-inflammatory cytokines: IL-12, IL-6, IFNγ, IL-2) and liver-induced damage associated (transaminase levels) compared to control groups. A local immunosuppressive effect was also observed after treatment with significant inhibition of inflammation and fibrosis associated genes (RNA level). We have confirmed IL35 mRNA therapy efficacy in the CYP2D6 chronic model characterized by autoreactive T/B cells and secretion of anti-CYP2D6 autoantibodies. IL35 mRNA treatment decreases significantly secretion of anti-CYP2D6 autoantibodies compared to control groups. Importantly, total IgG antibodies level was not affected showing the specific effect of IL-35 on activated autoimmune T cells. Altogether, our results illustrate that localized IL-35 expression, through the use of mRNA/LNP therapy, has the potential to reverse acute/chronic hepatitis inflammation and open new perspectives to use this novel mRNA therapeutic strategy for treatment of multiple auto-immune or inflammatory diseases.

Basic Science of Immunology - Adaptive Immunity

Th129. Theoretical and Practical Considerations for Validating Antigen-specific B Cell Immunospot Assays

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Abstract Text: Owing to their ability to reliably detect even very rare antigen-specific B cells in cellular isolates such as peripheral blood mononuclear cells (PBMC), and doing so robustly in a high throughput-compatible manner, B cell ELISPOT/FluoroSpot (collectively “B cell ImmunoSpot”) tests have become increasingly attractive for immune monitoring in regulated settings. Presently, there are no guidelines for the qualification and validation of B cell ImmunoSpot assay results. Here, we propose such guidelines, building on the experience acquired from T cell ImmunoSpot testing in an environment adhering to the requirements of regulatory bodies yet taking the unique features of B cell assays into account. A streamlined protocol is proposed that permits the performance of all tests needed for the formal validation of an antigen-specific B cell ImmunoSpot assay in only three experiments, utilizing 2.2 x 107 PBMC per donor. Subsequently, utilizing only 1-2 x 106 PBMC per sample (obtainable from 1-2 mL of blood), a validated multiplexed assay enables accurate quantification of the frequency of antigen-specific memory B cell-derived blasts secreting IgM, IgG, IgA or IgE antibodies. Collectively, such multiplexed B cell ImmunoSpot assays offer immense value for B cell immune monitoring programs due to their ease of implementation, scalability, applicability to essentially any antigenic system, economy of PBMC utilization, and last but not least, the high content information gained.

Th130. Granzyme K Drives a New Pathway of Complement Activation That Contributes to Disease Pathology

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Abstract Text: Granzyme K (GZMK) expressing CD8$^+$ T cells have recently been defined as the predominant CD8$^+$ T cell population in inflamed tissues in multiple autoimmune diseases. However, the overall function of GZMK has been relatively undefined. Here, we show that GZMK is a novel activator of the complement cascade. GZMK cleaves C2 to C2b and C4 to C4b to form an active C3 convertase that cleaves C3 into bioactive C3a and C3b. GZMK further forms active C5 convertases that generate C5a and the membrane attack complex, thus generating all key components of the complement cascade. GZMK is continuously released from CD8$^+$ T cells in an antigen-independent manner. Upon release, GZMK binds to negatively charged surface molecules through electrostatic interactions, and membrane bound GZMK is more efficient at forming C3 convertases. In human rheumatoid arthritis synovium, we find evidence of increased complement activation near GZMK$^+$ cells. Importantly, in a model of inflammatory arthritis, Gzmk$^{-/-}$ mice have less severe joint swelling than Gzmk$^{+/+}$ or Gzmk$^{+/-}$ mice. In a second disease model, imiquimod-induced dermatitis as a model of psoriasis, Gzmk$^{-/-}$ mice have less severe erythema, scaling and thickening of skin compared to Gzmk$^{+/+}$ or Gzmk$^{+/-}$ mice. Strikingly, Gzmk$^{-/-}$ mice have markedly less C3d and C4d, products of complement pathway activation. These data suggest GZMK is driving the activation of the complement cascade *in vivo*. Taken together, we define a new pathway of complement activation that is dependent on GZMK and has the potential to drive inflammation in a variety of tissues and disease states.

Th132. Reproducible Single Cell Annotation of Programs Underlying T Cell Subsets, Activation States, and Functions

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Abstract Text: T cells recognize antigens and induce specialized gene expression programs (GEPs) enabling functions including proliferation, cytotoxicity, and cytokine production. Traditionally, different classes of helper T cells express mutually exclusive responses – for example, Th1, Th2, and Th17 programs. However, single-cell RNA sequencing (scRNA-Seq) experiments have revealed a continuum of T cell states without discrete clusters corresponding to these subsets, implying the need for new analytical frameworks. Here, we advance the characterization of T cells with T CellAnnoTator (TCAT), a pipeline that simultaneously quantifies pre-defined GEPs capturing activation states and cellular subsets. From 1,700,000 T cells from 700 individuals across 38 tissues and five diverse disease contexts, we discover 46 reproducible GEPs reflecting the known core functions of T cells including proliferation, cytotoxicity, exhaustion, and T helper effector states. We experimentally characterize several novel activation programs and apply TCAT to describe T cell activation and exhaustion that predict response to immune checkpoint inhibitors across multiple cancer types. Our software package starCAT similarly enables reproducible annotation of other cell types and tissues.

Th133. ScRNA-Seq Profiling of Antigen Specific CD4$^+$ T Cells in Semi-Allogeneic Transplantation and Pregnancy

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Abstract Text: In pregnancy, the semi-allogeneic fetus is afforded immunological tolerance despite expression of paternal alloantigens. In contrast, full or semi-allogeneic allograft transplantations without immunosuppression succumb to acute rejection. While many studies have focused on $CD8^+$ T Cells in both transplant and pregnancy models, there is a knowledge gap of $CD4^+$ T cell biology at the single cell level. We performed scRNA sequencing on endogenous, donor-specific $CD4^+$ T cells to compare their fate in tolerance induced by pregnancy or co-stimulation blockade to allogeneic heart grafts. We also compared donor-specific $CD4^+$ T cells from skin and heart allograft rejection as well as from pregnancy after skin sensitization to model the intersectionality between pregnancy and transplantation. Donor- specific (based on 2W:I-Ab tetramer-binding) $CD4^+$ T cells from spleen and lymph nodes were sorted and sequenced. We defined shared and distinct unbiased clusters, differentially expressed genes, and pregnancy/transplant specific pathways in donor-specific $CD4^+$ T cells elucidating conserved and divergent gene signatures of tolerance, sensitization, and rejection. Specifically, we leverage our transplant and pregnancy models to report novel heterogeneity in follicular and regulatory T Cells at the single cell level.

Th134. Single Cell Transcriptomics in the Early Childhood Duodenum Reveals Resting and Active Mucosal Mast Cells

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Abstract Text: Tissue-resident mast cells (MC) are key sensors and effectors of type 2 immune responses and are important in allergic inflammation. Despite their demonstrated role in inflammatory responses, there has been relatively limited molecular characterization of resident MC in healthy gut tissues. To address this, we analyzed single-cell RNA-seq data of histologically normal duodenal biopsies collected as part of the multi-center Gut-AGE project (n=88 children, aged 7.9 months to 12 years), from Boston, Mississippi, Virginia and Pakistan. Duodenal biopsies were collected and cryopreserved for single-cell RNA sequencing. Biopsies were dissociated using an optimized single-fraction protocol and multiplexed for droplet-based single cell sequencing producing a total of 226,706 individual cells in the whole dataset. A mast cell data object (Seurat) was created and analyzed (n=476 individual mast cells). Seurat analysis and rxtatics packages were utilized to evaluate mast cell populations in participant samples. Cellular pathways were analyzed using GO Enrichment analysis. This analysis revealed two distinct MC clusters: active and resting. The active MC cluster demonstrated upregulation of genes associated with MC degranulation and activation including SIGLEC6, CDK15, ST8SIA6, ENPP3, JAK1 and STAT3. This data demonstrates for the first time that resident MCs exist in both active and resting states in the non-inflamed human pediatric duodenum and the relationship of these cell-states may be important in maintaining mucosal immune homeostasis.

Th135. Stimulated $CD4^+$ T Cells Initiate Blood Coagulation Through Polyphosphate Release

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Abstract Text: Chronic inflammatory diseases are associated with an increased risk for thrombosis, but the contribution of T cells to activate coagulation factors is poorly understood. We found that $CD4^+$ T cell stimulation promotes the generation of inorganic polyphosphate (polyP), that links phosphate units by phosphoanhydride bonds to form long biopolymers. Corresponding to its intracellular accumulation, polyP levels increased on the surface of stimulated T cells and triggered the activation of coagulation factor XII in chromogenic cleavage assays. Moreover, the addition of stimulated, but not naive, $CD4^+$ T cells increased thrombin generation and decreased clotting time in a polyP-dependent manner in murine whole blood thrombography and mechanical coagulometer analysis, respectively. In line with the pro-coagulant role *in vitro*, we found that systemic T cell stimulation promotes thrombus formation in a venous thrombosis model *in vivo*. In human blood, flow cytometric analysis revealed that membrane-bound polyP increases on the surface of CD62L-$CD44^+$ effector memory T cells. Furthermore, elevated polyP levels were found on $CD4^+$ T cell populations that react specifically against corona virus SARS-CoV-2-derived peptides in comparison to cells restimulated with cytomegalovirus-derived peptides or *de novo* stimulation with superantigen, indicating that antigen-dependent differentiation delineates polyP release by T cell subsets. In summary, we found that stimulated T cells contribute to polyP-dependent FXII activation that initiates thrombus formation via the intrinsic coagulation pathway. Our results provide new insights into T cell-driven immunothrombosis and may help to elucidate the increased risk for thrombosis that is associated with chronic inflammation and virus infections.

Th136. The CLOCK Protein Regulates Naive $CD4^+$ T Cell Differentiation in Mesenteric Lymph Nodes Independently of BMAL1

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Abstract Text: Immune cells secrete cytokines and chemokines rhythmically in response to the circadian clock, regulating time-of-day-dependent immune cell trafficking, antigen presentation, and host responses to infections. These rhythms are driven by core clock genes, including Clock and Bmal1. However, their roles in naive CD4 T cell differentiation remain unclear. Our data indicate IL-17 cytokine expression by gut infiltrating T cells is circadian regulated. Mice expressing a dominant negative Clock mutant (ClockD19) are inflamed and have more Th17 cells compared to B6 and $Bmal1^{-/-}$ mice. We mapped chromatin accessibility, Clock/Bmal1 binding, and the expression profiles of naive $CD4^+$ T cells from mesenteric lymphnodes of ClockD19 and B6 mice. Chromatin accessibility was reduced in ClockD19 T cells, consistent with RNA-seq data indicating gene downregulation, including key circadian genes. Interestingly, ClockD19 T cells upregulated Hes7, a key factor in Notch signaling pathway and deregulated heat shock response genes. Furthermore, ClockD19 cells upregulated Wnt signaling genes and oncoproteins like Mycl, which implicated in cell growth and Th17 differentiation. ChIP-seq analysis revealed, in wild-type mice Clock and Bmal1 bind to genes associated with cell cycle, RNA metabolism and protein folding. However, in ClockD19 mice they bind to the promoters of genes involved in IL-17 signaling. Focusing exclusively on Bmal1-bound genes does not support a role in Th17 differentiation. However, Clock appears to regulate Th17 differentiation and influence naive $CD4^+$ T cell fate independently of Bmal1, and mutations like ClockD19 deregulate this process, skewing differentiation toward Th17 cells. Ongoing research aims to uncover additional mechanisms by Clock/Bmal1 influence the naive $CD4^+$ T cells fate.

Th137. The High-Throughput CyTOF XT PRO Mass Cytometer Enables High-Dimensional Immune Profiling with High Sensitivity, Repeatability and Reproducibility

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Abstract Text: Reproducible immune profiling is crucial for translational and clinical research and developing accurate prognoses and effective treatments. Reproducibly identifying low-expressing targets and rare functional populations is challenging with fluorescence flow cytometry due to spectral spillover, autofluorescence and unmixing errors, issues not present with mass cytometry. Further, multiple workflows in mass cytometry provide flexibility and minimize technical variation, including the abilities to freeze metal-tagged antibody cocktails, barcode samples and freeze stained samples for future acquisition. To assess the consistency of mass cytometry staining and acquisition workflows, human whole blood and PBMC were stained with antibody panels containing up to 50 surface and cytoplasmic targets. Samples were frozen and acquired using CyTOF™ XT PRO and CyTOF XT systems on a later date. The CyTOF XT PRO system allows for up to 4x increased acquisition rate compared with the CyTOF XT system. Both systems were highly repeatable and reproducible across multiple instruments. Cell population frequencies and signal intensities were not significantly different between the two CyTOF systems. Both systems had high sensitivity and dynamic range for abundant and low-abundance markers to accurately identify T helper cell populations. Overall, these studies demonstrate that CyTOF XT and CyTOF XT PRO systems generate highly repeatable and reproducible data, and the 4x increased event rate of the CyTOF XT PRO system does not compromise data quality. Automated acquisition by CyTOF systems enables researchers to accurately and reproducibly streamline immunophenotyping and functional profiling, leading to accelerated biological insights. For Research Use Only. Not for use in diagnostic procedures.

Th138. Tumor-Infiltrating Regulatory T Cells Are Thymus-Derived

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Abstract Text: The presence of FoxP3 regulatory T cells (Treg) in the tumor microenvironment has been associated with poor clinical outcomes. However, the nature of the infiltrating Tregs, particularly whether they represent thymic-derived Tregs or CD4 T cells that undergo a Treg differentiation process locally, remains an open question. The aim of this work was to determine whether CD4 T cells specific to a tumor-derived antigen become FoxP3$^+$ Tregs in the tumor microenvironment or in tumor draining lymph nodes. B16 murine melanoma cells that expressed ovalbumin (B16-OVA) or not (B16) were inoculated into CD45.1$^+$ wild-type B6 mice. Naive CD4$^+$ CD45.2$^+$ Foxp3GFP OT-II cells (specific for OVA) were adoptively transferred. CD4 T cell migration into the tumor and tumor draining lymph nodes was analyzed. FoxP3 expression in transferred OVA-specific cells was quantified. Migration of adoptively transferred OT-II cells into the tumor was negligible, disregarding whether the tumor expressed OVA or not. Virtually all FoxP3 CD4 T cells found in the tumor represented endogenous Helios$^+$ thymic-derived Tregs. OT-II cells recovered from tumor draining lymph nodes were mostly FoxP3 negative. These results were not affected by the capacity of the mouse or the tumor to produce TGF-beta. These results suggest that the regulatory T cells that infiltrate murine melanoma are thymic-derived and that local differentiation of FoxP3$^+$ regulatory T cells from naive CD4 T cells is negligible, even when CD4 T cells express a TCR able to recognize a tumor-derived antigen with high affinity.

Tu143. Antigenic Variation in Primary Exposure to SARS-CoV-2 Can Elicit Different Public Antibody Classes in Macaques

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Abstract Text: Continued immune escape by newly evolving SARS-CoV-2 variants has led to concerns that antigenic imprinting toward earlier variants may limit the efficacy of updated vaccines. However, most studies to date have examined this phenomenon primarily via serum reactivity and/or phenotypic characterizations of memory B cells. Here, we leverage a previous study of homologous versus heterologous boosting in nonhuman primates (NHPs) to investigate the immunogenetic underpinnings of imprinting. Two groups of NHPs were immunized with the clinical mRNA-1273 primary vaccine regimen and then boosted 6 months later with either mRNA-1273 (homologous) or mRNA-1273.β (heterologous). A third group received a primary vaccine series using mRNA-1273.β without boosting. All groups were then challenged with a β-variant virus. We used variant-specific antigenic probes and single-cell sequencing using the 10X Chromium platform to assess the longitudinal development of cross-reactivity in all three cohorts. Notably, we found a specific increase in the usage of heavy chain V gene IGHV3-ABA in the group primed with mRNA-1273.β. When paired with IGLV3-AED, these antibodies bound to an epitope in RBD. Most were able to weakly bind to and neutralize Omicron variant BA.1 and two demonstrated modest neutralization even against modern Omicron variants JN.1, KP.2, and KP.3. Negative-stain electron microscopy was used to understand the structural basis for imprinting. Overall, we show how different primary exposures can impact the immunogenetic characteristics of the antibody response, thus driving antigenic imprinting.

Tu144. Autophagy-related Lipid Kinase vps34 Is Required for B Cell Homeostasis and Humoral Immunity

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Abstract Text: Autophagy is a highly conserved degradation process that delivers cytoplasmic products to lysosomes for recycling. The lipid kinase Vps34/PIK3C3 is vital for autophagosome formation, endosomal sorting, and phagocytosis. We studied mice with a Vps34 ablation either conditionally in pro-B cells with CD19-Cre (Vps34ΔB mice) or inducibly in mature B cells by huCD20-CreERT2 (Vps34iΔB mice). Vps34ΔB mice showed reduced numbers of follicular and marginal zone B2 cells and a major defect in peritoneal B1 cells. LPS-stimulated splenic B cells exhibited lower mitochondrial respiration, more ROS, and increased expression of IL-10. These mice developed varied responses to distinct T cell-dependent (TD) immunization, with either enhanced or modestly reduced total or antigen-specific germinal center (GC) B cells. However, tamoxifen-treated Vps34iΔB mice exhibited major defects to TD immunization, indicating that early Vps34 depletion endows the resulting B cells with advantages in primary responses. Lastly, B cells from Vps34ΔB mice exhibited reduced expression of MHC II-bound CLIP and, surprisingly, elevated MHC II-restricted presentation of cytosolic but not membrane-bound self-antigens. Thus, we propose that Vps34-deficient B cells exhibit mitochondrial stress with accumulation of ROS, resulting in an anti-inflammatory cytokine profile, defective B cell homeostasis, and altered immune responsiveness. These studies will inform the development of B cell-based vaccines and immunotherapies.

Tu145. Characterisation of the Immune Response to EBV Infection in Tonsil Organoid Cultures

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Abstract Text: Despite the global health and economic problems that Epstein Barr Virus (EBV) poses, there is no licensed prophylactic vaccine. A better understanding of protective immunity against EBV would be easier if more were known about the immunological and virological events that occur during primary infection. However, for obvious reasons, we are almost completely ignorant about the earliest phases of the virus-host interaction. Since the initial phases of both EBV infection, and the immune response to EBV occur in the oropharynx where tonsils are located, we have undertaken a high-resolution study of the very first phases of virus infection and the immune response to that infection using a physiologically relevant model: EBV infection of tonsil organoids. Strikingly, EBV replication is efficiently controlled in tonsillar tissue from seropositive children , whereas EBV transformed cells are readily isolated after infection of tonsils from seronegative subjects. We have analysed the humoral, cellular and cytokine/chemokine responses in these organotypic cultures and characterised key features influencing control or susceptibility to EBV infection of B cells in these cultures. The implications of this work for vaccine development will be discussed.

Tu146. Deficiency of TLR7 and IRF7 Impairs the Adaptive Immunity in Respiratory Viral Infection

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Abstract Text: Emerging clinical evidence suggests that the loss of X-linked recessive toll-like receptor 7 (TLR7) attributes to 2% of men under 60 years old with severe COVID-19, highlighting the critical pathogenic role of TLR7 in an innate immunity against SARS-CoV-2 infection. Previous studies also indicate that TLR7 contributes to adaptive immunity, namely antibody production. Recently, we reported that deficiency of Tlr7 and Irf7 in mice impairs innate and adaptive immunities against SARS-CoV2 infection associated with the reduced interferon (IFN) alpha and gamma levels (PMID: 39289468). However, the exact cellular and molecular mechanisms underlying TLR7-mediated signaling events that contribute to the immunity against the respiratory viral infection including Flu infection remain unclear. To dissect this question, we used the compound mice specifically deficient in IFN alpha receptors on dendritic cells (DC) (IFNARloxP$^{+/+}$Cd11cCre$^{+/-}$). We demonstrated SARS-CoV-2 -infected IFNARloxP$^{+/+}$Cd11cCre$^{+/-}$ mice showed a tendency of lower anti-S IgG production than IFNARloxP$^{+/+}$Cd11cCre$^{-/-}$ mice at 14-day post infection (DPI) (p=0.1000, n=3). Furthermore, upon challenge with influenza virus H1N1 A/PR/8/34, infected Irf7$^{+/+}$ mice showed higher anti-Hemagglutinin (anti-HA) IgG levels than the infected Irf7$^{-/-}$ mice at 7 DPI (p=0.0292), and Tlr7$^{+/+}$ mice had higher anti-HA IgG levels than the infected Tlr7$^{-/-}$ mice at 21 DPI (p=0.0413). Together, these findings highlight the important roles of TLR7, IRF7, and IFN in orchestrating effective antibody production during respiratory viral infections. Further understanding these molecular pathways will enhance our knowledge of immune responses and could provide therapeutic strategies against RNA viruses.

Tu147. Design and Characterization of Anti-DNP Antibodies for Distinct Complement Engaging Properties for Cryo-EM Studies

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Abstract Text: Tailoring of IgG antibodies for specific and improved effector functions is crucial to secure optimal targeted treatment of diseases. We recently reported on an IgG Fc-engineering approach, the REW Fc technology, which is based three amino acids substitutions in the Fc that improves the pharmacokinetic properties of IgG antibodies due to enhanced pH dependent binding to the human form of the neonatal Fc receptor (FcRn). However, this technology also enhances the ability to recruit and activate the complement system via a mechanism that is postulated to be via increased Fc-Fc clustering upon antigen binding. Naturally, we have four human IgG subclasses with very distinct ability to engage different effector molecules, such as the complement system. As such, which IgG subclass to be used as a scaffold to build a therapeutic will depend on the need for which effector function that should be activated to secure efficient therapy while at the same time consider the potential for induction of severe side effects. Thus, in this project we aim to gain an in-depth understanding of how the REW Fc technology is behaving in the context of the four IgG subclasses, designed with specificity for the hapten DNP (dinitrophenol), by bridging biochemical assays with cryogenic electron microscopy (cryo-EM) studies where both Fc-Fc clustering and the ability to engage the complement system will be addressed. A detailed structural understanding will guide further engineering strategies with the purpose to develop IgG antibodies with fine-tunned complement activation capacity.

Tu148. Effects of Parathyroid Hormone in Peripheral B Cell Tolerance

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Abstract Text: Introduction: Hormones like prolactin and estrogens influence transitional B cells by promoting activation, rescuing them from apoptosis, and enhancing their survival. This immune modulation can contribute to loss of tolerance and the emergence of autoreactive B cells. While these hormonal effects are well-studied, the impact of parathyroid hormone (PTH) on transitional B cells remains poorly understood. Methods: To investigate the effects of PTH on B cell peripheral tolerance, we used the transitional mouse B cell line WEHI-231. Cells were stimulated with increasing concentrations of PTH for 24–96 hours, stained with Annexin V/PI or MHC II antibodies, and analyzed by flow cytometry. Results: Stimulation with high concentrations of PTH (32 and 64 ng/uL) significantly increased WEHI-231 viability at 72 and 96 hours compared to controls, with no similar effect observed at lower concentrations. This viability enhancement was absent in the mature B cell line A20. Furthermore, high PTH levels rescued WEHI-231 cells from anti-IgM-induced apoptosis at 48 and 72 hours and significantly increased MHC class II expression after 48 hours. All findings were statistically significant ($p < 0.05$). Conclusion: Our data propose that PTH may rescue transitional B cells from apoptosis, enhancing their survival. Additionally, the observed activation of transitional B cells with increasing PTH concentrations *in vitro* highlights a potential role in loss of tolerance and autoimmunity.

Tu214. Optimizing PBMC Utilization and Cryopreservation for B Cell ImmunoSpot® Analysis

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Abstract Text: Measuring frequencies of antigen-specific memory B cells (Bmem), their immunoglobulin (Ig) class and subclass usage, cross-reactivity and affinity can provide insights into the efficacy of future antibody responses in case of antigen re-encounter. B cell ImmunoSpot® assays can provide such information; however, like most cell-based tests, they too require considerable amounts of blood to be drawn from the test subject and this has hindered

their inclusion into clinical trials, and into routine immune diagnostics. Here, we introduce strategies for reducing the cell numbers required to less than 2 million peripheral blood mononuclear cells (PBMC) per antigen, obtainable from 2-3mL of blood. We establish that testing PBMC in singlet wells, but in serial dilution, enables more reliable and accurate Bmem frequency assessments than testing replicate wells at a single fixed cell number; except when Bmem frequencies are very low. We further show that these assays can be multiplexed for measuring four Ig classes, or IgG subclasses, without any appreciable loss in sensitivity. Lastly, we establish that PBMC can be cryopreserved in smaller numbers than the standard 10 million PBMC per vial and retain indistinguishable functionality. Collectively, the predictable need for and recovery of PBMC facilitates optimal cell utilization in downstream B cell ImmunoSpot® assays.

Tu216. AID Governs Plasma Cell Fate Decision by Demethylation of the Irf4 Locus in Germinal Center B Cells

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Abstract Text: Activation-induced cytidine deaminase (AID) is essential for B cell affinity maturation. We investigated why AID deficiency in human and murine models gives rise to giant germinal centers using the AID-R112H mouse model that is devoid of AID activity. The increased germinal center response was associated with accumulation of GC B cells predominantly in the light zone in immunized AID-R112H mice. AID-R112H GC B cells had reduced capacity to up-regulate IRF4 to initiate plasma cell differentiation, leading to accumulation of a transitional germinal center population with reduced GL7 expression. Genetic introduction of a high affinity B cell receptor (BCR) was unable to restore plasma cell differentiation of AID-R112H B cells while ectopic expression of catalytic active AID in AID-R112H B cells rescued plasma cell generation. AID-R112H disrupted the interaction with the Ten-eleven Translocation 2 (TET2), leading to impaired recruitment of AID to the Irf4 promoter/enhancer. Consequently, DNA demethylation at the Irf4 promoter/enhancer was hindered, thereby impeding the Irf4 expression in GC B cells for commitment to the plasma cell lineage. This data reveals a B cell-intrinsic mechanism that governs the plasma cell fate decision through epigenetic remodelling mediated by AID.

W154. A Multiomics Resource of B Cell Activation Helps Decipher Disease Pathogenesis

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Abstract Text: Most genetic variants that confer risk of complex immune-mediated diseases (IMD) affect gene regulation in specific cell types. Their target genes and focus cell types are often unknown, partially because some effects are hidden in untested cell states. B cells are drivers of IMDs such as lupus and multiple sclerosis. Yet, B cell activation states are underrepresented in functional genomics studies. In this study, we obtained B cells from a total of 26 healthy female donors. We stimulated B cells *in vitro* by targeting key pathways in six activation conditions

such as the CD40, BCR, TLR7, TLR9, and a cocktail that promotes differentiation into double negative 2 (DN2) IgD-CD27- CD11c+ B cells. We profiled B cell activation at different time points using RNA-seq, single-cell RNA-seq coupled with surface protein markers (CITE-seq), and ATAC-seq. We provide an in-depth characterization of how IMD-associated genes respond to stimuli and group into modules with distinct functions. Using single-cell data, we describe pathway-dependent B cell fates. Chromatin data reveal the transcription factors involved in pathway-dependent B cell activation. These open chromatin regions capture a significant proportion of the genetic risk of IMDs and elucidate IMD genetic risk variants with previously unknown function. These data are shared via an interactive browser that can be used to query the dynamics of gene regulation and B cell differentiation during activation by different stimuli.

W155. Evidence for Human Thymopoiesis in a Porcine Thymus Graft and T Cell Tolerance to the Source Pig in a Living Human Recipient of a Porcine Galsafe™ Thymokidney Xenotransplant

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Abstract Text: Introduction: Porcine thymic transplantation tolerizes xenogeneic recipients to the porcine source animal in animal models (1,2). A 54-year-old woman with complex comorbidities received a composite thymokidney transplant from a GGTA knockout pig (GalSafe™), achieving initial renal function before graft failure for non-immunological reasons on POD47. Methods: Serial PBMC samples were analyzed by spectral FCM, and mixed lymphocyte reactions (MLRs) were performed on purified T cells to assess tolerance. Thymocytes were isolated from the thymic portion of the graft explant and analyzed using spectral FCM. Results: T cell levels in peripheral blood declined to 10/µl post-induction therapy and recovered to a peak of 342/µl by POD28. By POD21, 64% of the recovering CD4 T cells displayed a CD45RA+CCR7+ "naive" phenotype and expressed CD31, consistent with recent thymic emigrants (RTEs). Pre-transplant CD4 T cells included < 10% RTEs. Cells from the POD47 thymic graft contained human CD45+ leukocytes (0.85%), including double-positive (CD4+CD8+), single-positive, and double-negative thymocytes. The double-negative cells expressed markers such as CD5, CD7, CD1a, and CD34, consistent with human T cell progenitors in the porcine thymus. MLR results demonstrated progressive hyporesponsiveness toward the source pig with preserved responses to third-party pig and allogeneic human cells, achieving full source-specific unresponsiveness by POD47 and POD86. Conclusion: These findings suggest that a porcine thymus in a GGTA kncockout thymokidney graft supported human thymopoiesis and the development of T cells tolerant to the source pig. Thymic transplantation is a promising approach to achieving T cell tolerance in xenotransplant recipients.

W156. Expression and CD47-Independent Function of SIRPG: A Primate T Cell-Specific Gene

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Abstract Text: SIRPG is an immunoglobulin-like transmembrane protein expressed almost exclusively in primate T cells. Genetic variations in SIRPG have been linked to increased risk of type 1 diabetes and its level in tumor infiltrating lymphocytes is prognostic in several cancers. Its only known ligand is CD47, however, its function and how its expression is regulated remain poorly understood due to its absence in rodents. Here, we show that SIRPG expression is downregulated in effector T cells and is almost mutually exclusive with Granzyme B expression in

resting peripheral T cells. Its expression is induced by anti-CD3 stimulation and further enhanced by the TNF-α inhibitor adalimumab but not certolizumab. Ablation of SIRPG has little impact on the activation and production of IFNg or TNFa of T cells but lead to impaired expression of several mitosis-regulating genes, including UBE2C and CDK1, resulting in less robust proliferation, and a subset of activation-induced co-receptors, such as CRTAM, SEMA4a, TNFRSF9, and TNFRSF18. Interestingly, SIRPG and its down-stream genes are enriched in a unique cluster of melanoma-infiltrating exhausted CD8 T cells expressing a high level of cell cycle genes. Mechanistically, the SIRPG-mediated proliferation and expression of CRTAM is cell autonomous and independent of CD47. Structural and functional analyses will be carried out to investigate the role of its extracellular and cytoplasmic domains. Collectively, our findings provide novel insights into the regulation, function, and mechanism of action of SIRPG in T cells, with potential implications for modulating the T cell response in various human diseases.

W157. Expression of the Co-receptors TIGIT and CD226 on Tregs and Their Role in Autoimmunity

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Abstract Text: TIGIT is an important co-inhibitory receptor on Tregs, which are crucial for maintaining tolerance and preventing autoimmunity. Sharing the same ligands (CD155/CD112), the effect of co-stimulatory CD226 on Tregs is less well defined, with $CD226^+$ Tregs linked to worsened disease activity in various inflammatory conditions. Here, we investigated the co-expression of TIGIT and CD226 on Tregs in T cell activation and autoimmunity. Through spectral flow cytometry analysis, we observed increased frequency of $TIGIT^+CD226^+$ Tregs in the inflamed joint (synovial fluid, SF) than blood (PB) Tregs from individuals with Juvenile Idiopathic Arthritis. This suggests co-expression of these co-receptors could represent altered Treg response to certain inflammatory stimuli. Indeed, $TIGIT^+CD226^+$ Tregs from both SF and PB displayed a memory phenotype with a heightened activation state, through increased expression of CD71, 4-1BB, PD-1 and CD39. *In vitro* expression kinetic assessment of TIGIT/CD226 post-activation revealed higher co-expression on Tregs than Tconv, with maintenance of $TIGIT^+CD226^+$ Treg co-expression upon further stimulation. $TIGIT^+CD226^{hi}$ Tregs had decreased Ki67 expression after seven-day expansion, suggesting a terminal differentiated state with altered proliferative ability. Cell sorting on $TIGIT^-CD226^-$ suggested that Treg phenotype is dependent on which co-receptor is upregulated first, with $TIGIT^-CD226^{hi}$ populations predominantly expressing ICOS with altered cytokine production. We therefore propose $TIGIT^+CD226^+$ co-expression represents a highly activated but terminally differentiated Treg state in response to continuous inflammatory stimuli, such as in the inflamed joint. This state could represent altered functional stability of Tregs and present as a possible target in monitoring or restoring tolerance in autoimmunity.

W158. Human Neonatal CD8 T Cells Exhibit Rapid, Short-lived Effector Responses and a Unique Transcription Factor Landscape

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Abstract Text: Neonates are distinct in their clinical and cellular responses to viral infections, with neonatal CD8 T cells featuring innate-like characteristics and a lower threshold for T cell receptor (TCR) activation relative to adult counterparts. However, specific molecular programs that drive these unique responses are incompletely understood, particularly in humans, and targetable pathways to modulate viral illness in this vulnerable population remain to be elucidated. We set out to define the unique response characteristics and underlying transcription factor landscape of human neonatal CD8 T cells, which may be developmentally programmed for rapid responses to limit immunopathology in early life. Here, we report that human naive CD8 T cells from neonates are poised for rapid

effector differentiation, with elevations of KLRG1, KLRB1, FCER1γ, CD226/DNAM1, granzyme K, TNFα, IL-2, and glycolysis compared to naive CD8 T cells from adults. Further, rapid cell death occurs with or without activation of neonatal CD8 T cells, with improved survival in the presence of IL-2 and IL-7. These features are associated with a unique transcription factor landscape in neonatal CD8 T cells, including high baseline and post-activation expression of Thymocyte selection–associated high-mobility group box (TOX) and Helios (IKZF2). In conclusion, neonatal CD8 T cells have a unique transcription factor landscape associated with rapid differentiation into short-lived effector cells, revealing key nodes of regulation relevant to the distinctive immunobiology of human neonates.

W159. Identification of B Cell Epitopes of BIP and α-1 Giardin Proteins from Giardia Lamblia

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Abstract Text: Giardiasis is a global health problem caused by the parasite Giardia lamblia. Current treatments often lead to side effects and fail to prevent reinfections. Actually, there is no vaccine for human infections, highlighting the need to elucidate the immunological mechanisms triggered during infection to develop new therapeutic strategies. Our research group is investigating immunogenic proteins from G. lamblia as potential vaccine candidates, including BIP and α-1 giardin. The B- and T cell epitopes of these immunogenic antigens remain unknown. Therefore, the aim of this study was to identify regions within the BIP and α-1 giardin proteins that contain B cell epitopes. To achieve this, specific polyclonal antibodies against these proteins were generated by intraperitoneal immunization in Balb/c mice. Experimental data obtained by Western Blotting assays, combined with in silico analysis, revealed that the amino acid region 237(ILE)-511(ILE) of the BIP protein contains immunogenic B cell epitopes. Furthermore, specific B cell hybridomas targeting BIP were generated, allowing the purification of the monoclonal antibody 6H2.H7, which is currently being characterized. In the case of the giardin α-1 protein, immunogenic fragments were identified by western blotting and electrospray ionization mass spectrometry, determining that the amino acid sequence 15(ISO)-73(ASP) was immunogenic. Identifying these immunogenic regions in G. lamblia proteins is crucial for understanding the parasite's immunological interactions with its host. Furthermore, this information will guide the design and development of a multipeptide vaccine able of eliciting an effective immune response against the parasite.

W160. Identifying Therapeutic Vulnerabilities of B Cell Tumors Disseminating into Immune Privileged Sites

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Abstract Text: Diffuse large B cell lymphomas (DLBCLs) that infiltrate non-lymphoid tissues, including immune-privileged sites such as the brain, represent a critical clinical challenge, due to a lack of mechanistic understanding. BTG1 genetic alterations are found in up to 70% of DLBCLs with high frequency of non-lymphoid tissue infiltration and poor prognosis. We find that they are the top mutations in circulating tumor DNA from the cerebrospinal fluid of DLBCL patients with brain infiltration. Taking advantage of our unique Btg1Q36H mutant conditional mouse model, in which Btg1Q36H B cells outcompete wild-type cells through a faster response to T cell signals and facilitated Myc protein synthesis, and rapidly transform into disseminated B cell lymphomas; we set out to identify signals driving Btg1Q36H B cells' competitive fitness. We found that interleukin-21 (IL-21)-induced signaling is the top upregulated pathway in Myc-positive Btg1Q36H cells and is enriched in BTG1 mutant DLBCLs. This is associated with elevated

levels of IL-21 receptor (IL-21R) and a lower IL-21 threshold for downstream STAT3 activation. Btg1Q36H B cells show an IL-21- and T cell help-dose-dependent competitive advantage. Knocking down IL-21R rescues their advantage. These findings identify the IL-21-STAT3 axis as a critical driver of Btg1Q36H B cells' competitive fitness and potential vulnerability of derived disseminated DLBCLs. We further generated a mouse pre-clinical model of brain-infiltrating DLBCL, to test the efficacy of targeting this axis to kill brain-infiltrating tumor cells. Future translation is envisioned using IL-21-blocking antibodies and STAT3 inhibitors currently in clinical trials for other diseases.

W161. Immune Dysregulation in Children with down Syndrome: Altered Tonsillar Architecture and Reduced Follicle Size Correlate with Increased Frequencies of CD4$^+$ PD1int CXCR3$^+$ T Cells and Decreased Follicular Helper T Cell Frequencies

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Abstract Text: People with Trisomy 21 (T21) have distinctive susceptibility to diseases due to an early immune dysregulation. We investigated T-B cell interactions in tonsils, as a proxy of secondary lymphoid organs (SLO). We looked for the germinal center (GC) reaction, the area within follicles where mature cells undergo intense proliferation, differentiation, and selection during an immune response, with the help of follicular helper T cells (Tfh). Multiparametric flow cytometry and multiplex immunofluorescence characterized B and T cells in hypertrophied, non-infected tonsils in T21 children compared to age-matched controls (n=10). We focused on the expression of CXCR3 and other markers associated with Th1 response, inflammation and autoimmunity. The architecture and frequency of different populations from the tonsils' B and T zones were analyzed. There was a marked remodeling of T-B cell compartments in T21 tonsils, with increased frequency of different CXCR3-expressing cells, such as memory B cells ($p< 0.05$), GC B cells, plasmablasts ($p< 0.01$) and activated non Tregs nor Tfh CXCR3$^+$ T cells in detriment of conventional Tfh ($p< 0.05$). The density (#cells/mm2) of CXCR3$^+$ Tfh-like cells outside follicles significantly increased in T21, suggesting heightened extrafollicular responses. CXCR3$^+$ Tfh and B cells were closer together in the T zone of T21 tonsils, suggesting stronger interactions outside follicles. T21 substantially impacted the tonsil T and B cell compartments, inhibiting Tfh and GC formation while increasing CXCR3$^+$ B cells in the T cell zone. Our results suggest that both the GC reaction and he spatial distribution of T and B cells are altered in T21 tonsils.

W162. Immunomodulatory Effect of G. Lamblia Proteins in an Antigen Processing and Presentation Model *in vitro*

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Abstract Text: G. lamblia causes the gastrointestinal parasitosis giardiasis, colonizing the small intestine. In the intestine, antigens are continuously carried out by antigen-presenting cells (APCs), which endocytose and present peptides through their Major Histocompatibility Complex (MHC) molecules to T cells, promoting the detection of pathogens as well as favoring the induction of intestinal immune tolerance. During infection, G. lamblia secretes molecules that may modulate the host's response. Therefore, the aim of this study is to evaluate the potential immunomodulatory effect of G. lamblia in an antigen processing and presentation model *in vitro*. The model used was the APC (M12.C3.F6), hen egg lysozyme (HEL) as the antigen, and the T cell hybridoma 3A9, which is specific to HEL (epitope 48-61). Its activation promotes the secretion of IL-2 into the medium. To evaluate the activation of the 3A9 hybridoma, the IL-2-dependent CTLL2 cell line was used. Subsequently, the potential modulation exerted by a protein lysate from G. lamblia trophozoites was analyzed. The MTT assay was employed to analyze cell viability and proliferation. Our results showed that G. lamblia lysate induces inhibition in the processing and presentation

assay by reducing the activation of the 3A9 hybridoma, therefore, lowering the secretion of IL-2 to the medium. Additionally, the lysate concentrations use (12.5 and 25 ug/mL) did not affect the viability of the participant cells, confirming that the effect was due to the potential modulation of the proteins present in G. lamblia lysate. Future experiments aim to elucidate the possible modulation mechanisms conducted by G. lamblia proteins.

W163. Injectable Fatty Acid Nanovesicles as Next-generation Vaccine Adjuvant: integrating Physicochemical Characterization with Cytokine-mediated immunomodulation

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Abstract Text: Injectable docosahexaenoic acid (DHA) nanovesicles were formulated using film hydration method, characterized for their physicochemical and morphological properties. Transmission electron microscopy confirmed smooth, discrete structures with consistent morphology. The vesicles displayed uniform spherical shapes, high colloidal stability, optimal surface charge and good poly dispersity index. Thermal and structural stability were validated by differential scanning calorimetry, thermogravimetric analysis, X-ray diffraction, and nuclear magnetic resonance spectroscopy, supporting their suitability as injectable nano dosage form. Immunological studies in Wistar rats revealed that the DHA nano vesicles showed a stronger and more sustained immune response than the commercial hepatitis B vaccine. Elevated levels of pro-inflammatory cytokines, interleukin-6 (IL-6), interleukin-8 receptor (IL-8R), and interleukin-12 (IL-12) indicated activation of innate immunity and antigen-presenting cells. Simultaneously, upregulation of interleukin-2 (IL-2), IL-4, IL-10, and interferon-gamma (IFN-γ) reflected stimulation of adaptive immunity and immunological memory. Increased C-reactive protein (CRP) confirmed systemic immune activation, while stable immunoglobulin E (IgE) levels indicated no hypersensitivity. Overall, the DHA nanovesicle adjuvant system demonstrated an excellent vaccine adjuvant for enhancing hepatitis B vaccine immune response.

Basic Science of Immunology - Innate Immunity

Th139. Rhog Is Upregulated in Neutrophils and Monocytes Isolated from Peripheral Blood of Septic Patients and Animals and Regulates Migration and Inflammatory Functions in Innate Immune Cells *in vitro*

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Abstract Text: Rho GTPases RhoG is a regulator of cellular processes and plays a key role in modulating inflammatory responses. Despite its importance in cellular functioning and various diseases, the role of RhoG in the context of sepsis remains unexplored. Findings from the present study show that RhoG transcripts and protein were upregulated in human peripheral blood neutrophils, monocytes, and macrophages upon stimulation with Lipopolysaccharide (LPS), TNF-α, and chemokines *in vitro*. Higher RhoG expression was also observed in the neutrophils and monocytes isolated from septic mice and patients. Interestingly, the presence of fibronectin altered RhoG transcript expression, and blocking integrin β1 significantly reduced RhoG transcripts in inflammatory cells. Knockdown of RhoG significantly decreased the cell viability, which was restored in the presence of fibronectins. A significant reduction in differentiation and adhesion markers (e.g., CD14, MHC-II, CD11b, and integrin α4) and the production of proinflammatory cytokines (IL-1β and IL-6) was observed in primary monocytes. Knockdown of RhoG significantly increased cell spreading, migration, and velocity in both primary monocytes and differentiated HL-60 (neutrophil-like cells). Bioinformatic analysis linked RhoG to several proteins such as ELMO1/2, PIK3CB- AKT, DOCK1/2/5, and TRIO/KALRN. These findings indicate that RhoG plays an essential role in inflammatory response in sepsis by regulating immune cells and crosstalk with integrin β1.

Th140. The Role of Activin a in T Cell Mediated Modulation of Monocyte Function

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Abstract Text: Monocytes are key drivers of inflammation; therefore, understanding how these cells can be modulated is scientifically and clinically relevant. Healthy control peripheral blood-derived $CD14^+$ monocytes were cultured with activated autologous $CD4^+CD25^-$ effector T cells (Teffs) or $CD4^+CD25^+CD127^-$ regulatory T cells (Tregs). RNA-seq analysis revealed that Teffs and Tregs differentially altered the transcriptional signature of monocytes (n=11). The top upregulated gene in Teff-modulated monocytes was INHBA (Log2FC 7.5), encoding activin A, a pleiotropic cytokine present during inflammation. Furthermore, Teff-modulated $CD14^+$ monocytes displayed reduced mRNA and protein expression of the scavenger receptor CD36 (n=11) and showed reduced uptake of ox-LDL (n=6). Stimulation of monocytes with recombinant TNFα similarly reduced the expression of CD36 and uptake of ox-LDL, with an additive effect of Activin A. This further substantiates the modulatory effect of Activin A in the interaction between monocytes and Teff. Data mining of a pre-existing transcriptomic dataset of blood and joint-derived T cells from patients with rheumatoid arthritis showed the activin A receptors ALK4 and ACVR2A/B are present on Teffs. Flow analysis of $CD4^+$ T cells stimulated with activin A showed an increased frequency of TNFα-expressing T cells (n=5). The increase in TNFα-expressing cells was observed across different $CD4^+$ T cell subsets (IFN-γ^+, IL-10^+, IL-$17A^+$ and GM-CSF^+) suggesting activin A alters the polyfunctional profile of $CD4^+$ T cells. Together, these findings indicate a positive feedback loop, involving TNFα and activin A, between Teffs and monocytes resulting in impaired ox-LDL uptake. Supported by King's College London BHF Centre of Research Excellence [RE/24/130035] studentship to KJL and BBSRC studentship to AKD.

Th141. The Role of CXCR4-CXCL12 in the Migration and Development of Human Natural Killer Cells

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Abstract Text: Natural killer (NK) cells play a critical role in controlling viral infection and malignancy and undergo differentiation from multipotent progenitors in secondary lymphoid tissue. Despite dependence on the microenvironment for differentiation, little is known about the cell-cell interactions that promote NK cell trafficking and maturation in tissue. We have identified the chemokine receptor CXCR4 and its ligand CXCL12 as regulators of the cellular interactions between human NK cell precursors and stromal cells that support NK cell development. Using *in vitro* NK cell differentiation, we found that treatment of $CD34^+$ precursors with CXCR4 antagonist AMD3100 leads to a lower efficiency of NK cell generation. Since previous work demonstrates the association of cell migration with NK cell development, we interrogated the effect of AMD3100 on NK cell migration. Using live cell imaging, we found that AMD3100 treatment of NK cells or progenitor cells results in altered migratory properties; specifically, decreased arrest and adhesion of cells on $CXCL12^+$ stroma. We confirmed these findings in CXCR4 knockdown NK cell lines. Additional experiments using high resolution microscopy and flow cytometry suggest that integrins interact with CXCR4 in NK cells. High resolution imaging of pediatric tonsil by cyclic immunofluorescence demonstrates CXCL12 in tissue niches that likely support NK cell development. Our data suggests that the CXCR4-CXCL12 interaction is critical for migration and proper development of NK cells. These findings enhance our knowledge of the basic underpinning of NK cell biology and have critical implications for understanding human NK cell deficiencies and the development of NK cell therapies.

Tu149. A Novel 50-marker Intracellular Staining Panel Enables Unrivaled Detection of Functional Diversity Among Human Immune Cell Subsets

Laura Polanco[1], Michael Cohen[2], Erika Smith-Mahoney[1], Lauren Tracey[2], Christina Loh[2], Amedeo Cappione[2], Anna Belkina[1], David King[2] and Jennifer Snyder-Cappione[1]

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Abstract Text: The inflammatory functions of immune cells are well studied in clinical and translational immunology. However, the active anti-inflammatory and immunosuppressive axes have been grossly understudied; this gap negatively impacts discovery of therapeutic targets and drug development. Mass cytometry, an orthogonal platform for multidimensional single-cell analysis, avoids limitations of fluorescence-based cytometry such as signal spillover, autofluorescence and spectral unmixing data reduction. Understanding that mass cytometry would prove superior for detection of intracellular analytes and be ideal for use in downstream multivariate data analyses, we developed a 50-marker CyTOF™ panel with comprehensive profiling of immune subsets and 20-plus intracellular cytokines spanning major functional lineages. PBMC were stimulated using multiple conditions, stained and acquired on the CyTOF XT system. Datasets were analyzed using PhenoGraph clustering and visualized with opt-SNE to determine cellular diversity. Our findings showed a diverse array of functional signatures, including clear detection of IL-10- and IL-13-producing cells, and further revealed surprising co-expression patterns with the pro-inflammatory cytokine IFN. More significantly, we were able to detect expression of several secretory analytes not easily or previously identified by fluorescence cytometry, including the immunosuppressive cytokine TGF-. The CyTOF panel provides the widest visualization of functional diversity of human immune cells ever measured to our knowledge, and achieves this without additional controls, complicated panel optimization that occurs with large fluorescent panels or sacrificing signal resolution. Our work reveals novel immune regulatory mechanisms that may spur new avenues for significantly improving treatment of chronic inflammation and augmentation of vaccine responses.

Tu150. Characterization of Ibudilast Pharmacokinetics in the Rat After Intravenously Administration

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Abstract Text: Background: Ibudilast is a non-specific Phosphodiesterases (PDEs) inhibitor used for inhibiting PDE 3, 4, 10, and 11, which are involved in various cellular and biochemical processes. Previous studies demonstrated ibudilast carries many anti-inflammatory properties and might be a great therapeutic potential for various inflammatory diseases, including amyotrophic lateral sclerosis (ALS). However, there is a lack of information on the pharmacokinetics (PK) of the drug after intravenous (IV) administration, and an IV formulation is needed to determine if its immune inhibition improves organ failure outcomes for polytrauma injuries. Here, we sought to characterize the PK of ibudilast after IV administration in a healthy rat model. Method: Under general anesthesia, with prophylactic analgesics, 0.5 mg/kg ibudilast was delivered to a group of rats (n=4) via a single IV bolus. Plasma samples were collected at 0, 5, 10, 15, 30, 60, 120, 240, 480, 720, 1440 and 1920 minutes (mins) after drug infusion and quantified using mass spectrometry. Plasma PK parameters were calculated using the non-compartmental analysis (NCA) with PKanalix software. Results: The Cmax and Tmax of were 8.13±4.59 mM and 0.083 hour, respectively. The AUC0-t and AUC0-∞ were calculated as 5.20±2.86 mM*h and 5.52±3.08 mM*h. The overall clearance was 0.00067±0.00034 $L \cdot h^{-1} \cdot kg^{-1}$ with plasma half-life of 1.26±0.57 h (Mean±SE). The Vd was measured as 0.00088±0.00034 L·kg-1. Conclusion: Ibudilast seems to be quickly absorbed in blood after IV injection with a relatively short half-life. The PK data from this study will be used to develop an optimal dose of ibudilast for efficacy studies.

Tu151. Development of Novel Agonists for Human Inkt Cells

Ayaka Hara[1], Julia DiSapio[2], Morgan Coombs[1], Guzal Khayrullina[1], Vibhuti Joshi[1], Nikhila Bharadwaj[3], Md Alam[1], Hye Kim[1], Nargis Malik[1], Emily Steffke[1], Laila Latifi[1], John Hancock[1], Jo Spurgeon[1], Jenny Gumperz[3], Mark Gilbert[1], Amy Howell[2] and Masaki Terabe[1]

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Abstract Text: Invariant natural killer T (iNKT) cells are CD1d-restricted T cells expressing a semi-invariant T cell receptor (TCR) that recognizes lipid antigens and is involved in the anti-cancer immune response. Studies of a prototypic iNKT cell antigen, α-galactosylceramide (α-GalCer), analogs have shown that ceramide structure affects the activities of mouse iNKT cells. However, much less has been studied on human iNKT cells. This study evaluated the antigenicity of eight novel sulfatide-based lipids synthesized with fluorobenzene at the acyl chain terminus and three sphingoid base structures. Using human CD1d-tetramers loaded with sulfatide analogs, we examined the recognition of the sulfatide analogs by the TCR of human iNKT cells in peripheral blood mononuclear cells (PBMCs) from three donors. One analog we named SAP-3 was commonly recognized by all donors' iNKT cells. Sulfatide analogs activated iNKT cells measured by CD69 and CD25 induction in a CD1d-dependent manner. Cytokine analyses revealed donor-specific variability, with the commonly recognized analog SAP-3 inducing IL-2 production, unlike α-GalCer. To test the hypothesis that this analog SAP-3 enhances T cell activation and proliferation, we stimulated PBMC-derived CD8 T cells with influenza matrix protein-derived peptides and this analog SAP-3. The results showed that administration of this analog SAP-3 did not change the frequency of influenza-specific CD8 T cells. However, it increased the proportion of poly-functional CD8 T cells producing the three cytokines (IFN-γ, IL-2, and TNF-α) and two cytokines (IFN-γ and IL-2). These data suggested that the sulfatide analog SAP-3 could be used as a vaccine adjuvant inducing polyfunctional T cells.

Tu152. Exploring the Potential of Mycobacteria-derived Components in Anti-cancer Lymphocyte Activation

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Abstract Text: Natural Killer (NK) cell-based therapies are promising but require overcoming several challenges like their short lifespan and the difficulty to obtain large numbers of efficient anti-tumor cells. Recently, we have developed a novel strategy generating robust anti-tumor NK cells which proliferate over other peripheral blood mononuclear cells, after priming with Bacillus Calmette Guérin (BCG), followed by weekly stimulation with low doses of IL-12, IL-15, and IL-21 cytokines. These NK cells, which can expand up to 200-fold without the need of cell sorting, exhibit strong cytotoxicity against a wide variety of solid tumors. Notably, NK cell expansion was successful regardless of the mycobacteria's viability. Preliminary data have shown that mycobacterial extracts can enhance NK cell activation and degranulation against bladder cancer cells. Here, we describe that BCG subcellular fractions, or their combinations, could be used to enhance proliferation and anti-tumor function of NK cells and other innate lymphocytes, such as γδ T cells, depending on the BCG sub-strain used. Using three-dimensional (3D) cultures and organoid models, we further studied infiltration and solid tumor elimination by BGC-activated NK cells. We also explored the possibility of providing a more accurate donor selection using this preclinical testing model.

W164. Inflammatory Chromatin Accessibility in Monocytes of Patients Undergoing Elective Coronary Angiography with Severe versus Minimal Coronary Disease

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Abstract Text: Background: Experimental data suggest epigenetic changes prime monocytes and macrophages in cardiovascular disease (CVD), making them predisposed to hyper-inflammation even absent higher inflammatory gene expression. We hypothesized blood monocytes of stable outpatients with severe coronary artery disease (CAD) have more open chromatin at major inflammatory gene loci than those with none to minimal CAD with similar cardiometabolic risk factors. Method: We first performed single-cell ribonucleic acid and assay for transposase-accessible chromatin sequencing (scRNA-seq/scATAC-seq) on peripheral blood mononuclear cells, then bulk RNA-seq and ATAC-seq on sorted monocytes (N= 32). Primary analyses compared gene expression and chromatin accessibility at pre-specified inflammatory gene loci for those with severe multivessel CAD versus none to minimal CAD. Results: scRNA-seq/scATAC-seq analyses of severe CAD cases vs. CAD-free controls revealed monocyte-specific increased chromatin accessibility at IL1B in absence of higher IL1B expression for cases vs controls. Bulk RNA- and ATAC-seq in the larger cohort confirmed more accessible chromatin ($p< 0.05$ for all) for cases vs. controls around key inflammatory genes including IFNG, IL1B, IL10, and CCL2, in absence of higher expression of these genes. Additionally, mitochondrial oxidative phosphorylation genes NDUFB7, NDUFAF8, NDUFB, as well as several gene sets involved in oxidative stress (NCF1, CYBA, HMOX1) and angiogenesis/hypoxia response (VHL and HIF1a), are significantly repressed in cases compared to controls. Conclusion: Stable outpatients with severe CAD exhibit increased chromatin accessibility around key inflammatory gene loci in circulating monocytes in absence of overtly higher expression of these genes, suggesting an inflammatory imprint.

W165. Monocytes from Individuals with down Syndrome Demonstrate Evidence of TLR Signatures

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Abstract Text: Down syndrome (DS), or trisomy 21, is associated with immune dysfunction as evidenced by decreased vaccine responses, increased severity of viral infections, and increased incidence of certain autoimmune diseases, including Down syndrome-associated arthritis (DA). While studies have demonstrated evidence of dysregulated adaptive immunity in DS, there has been little focus on innate immune cells, which are key to setting the inflammatory tone. While recent work has shown that DS individuals have increased type I interferon (IFN) responses, due to the presence of the genes encoding subunits of the IFN receptor on chromosome 21, little work has examined innate immune receptor signaling in monocytes in DS. We isolated $CD14^+$ monocytes from individuals with DS (n=8) or age and sex matched healthy controls (HC, n=8), treated with agonists of TLR2, TLR4, TLR7 or TLR8, and identified the transcriptional signature using bulk RNA sequencing (RNA-Seq). We found that monocytes from individuals with DS had evidence of enhanced TLR signatures compared to HC at baseline, however DS monocytes had similar magnitude of gene induction after TLR agonist treatment. Additionally, these baseline differences were not explained by type I IFN responses. To learn more about DA and the role of monocytes in this disease, we performed single cell RNA-Seq on monocytes from individuals with DS without arthritis (n=7), DA (n=6), juvenile idiopathic arthritis (JIA) (n=6), or HC (n=8). Together, our data provide new insights into the innate immune responses of monocytes from DS subjects with implications for the refractory nature of DA.

W166. Novel Functional Diversity of Human T Cells Is Revealed as a Result of the Unprecedented Resolution of Intracellular Cytokines, Transcription Factors and Phosphoproteins Using Mass Cytometry (CyTOF XT)

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Abstract Text: Measuring functional signatures provides key insights into several facets of immunology, including T/B memory cell diversity following infection and/or vaccination and the panoply of immune activity in response to cell therapies, specifically the impact of inflammatory and immunosuppressive lineages on treatment efficacy. However, intracellular readouts using flow cytometry have been historically difficult to detect due to complications with spectral overlap and autofluorescence. We posited that mass cytometry may enable superior signal resolution within the cell compared with fluorescence cytometry. We evaluated three small (11–12-plex) clone-matched antibody panels using a spectral flow and mass cytometer. Each panel measured surface and intracellular analytes (cytokines and other markers of functional potential phosphorylation events and transcription factors); the fluorescent panels were designed to minimize spectral overlap. PBMC were stimulated, split, stained with fluorochrome- or metal-conjugated antibodies and acquired. Data was analyzed using PhenoGraph clustering and visualized with opt-SNE to determine cellular functional diversity. Most strikingly, stimulation-specific IL-10 and IL-13 positive cells were only detected using a CyTOF™ XT mass cytometer. Moreover, CyTOF technology provided cleaner signal-to-noise distinction for most phosphorylation events and transcription factors measured. Superior resolution translated to more accurate population clustering and a higher number of unique immune cell subset signatures, including clear detection of Tr1 and Tc2 populations. Overall, CyTOF technology uniquely provided a comprehensive picture of the immuno-diversity present in a sample; this platform will help illuminate swaths of currently "invisible" non-inflammatory immune responses, revealing new, broader classes of therapeutic targets and approaches to treat chronic inflammatory diseases.

W167. Platelet-mediated Neutrophil Extracellular Traps (NETs) Aggravate the Development and Progression of Diabetic Retinopathy

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Abstract Text: Background: Activated platelets and neutrophils are associated with the development and progression of type 2 diabetes mellitus (T2DM). However, platelet-neutrophil interactions in the pathogenesis of diabetic retinopathy (DR) are less explored. Therefore, we aimed to assess the role of platelet-mediated neutrophils extracellular traps (NETs) in the pathogenesis of DR. Methodology: Platelet activation, platelet-neutrophil aggregates (PNA), NETs, and circulatory level of inflammatory cytokines/chemokines were measured in healthy control (HC), T2DM, non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) subjects. Further, PNA, platelet-induced NETs, and NETs-induced Angiogenesis were assessed by *ex vivo* and *in vitro* studies. LC-MS/MS based SWATH analysis was performed to explore the molecular mechanism of platelet-induced NETs in DR. Results: Platelet activation, PNA, and NETs were significantly increased in T2DM, NPDR, and PDR compared to HC subjects. A positive correlation was found between platelet activation, PNA, and NETs. Furthermore, NETs were positively correlated with angiogenic and inflammatory markers (angiopoietin-2, ICAM-1, and IL-18). DR-platelets induced higher PNA and NETs compared to T2DM and HC-platelets. NETs induced by DR-Platelet induce angiogenesis in the RF/6A cell spheroids as compared to HC and T2DM platelet-induced NETs Proteomics analysis revealed that platelet-related proteins (CXCL7, WDR-1, vWF) were differentially expressed in DR and T2DM platelet-induced NETs compared to HC platelet-induced NETs. Conclusion: Our findings revealed that platelets get activated by a prolonged hyperglycemic environment, platelets further release inflammatory cytokines/chemokines

and aggregate with neutrophils and prime neutrophils towards NETs formation. NETs further promote angiogenesis and contribute towards the progression of DR. Keywords: Diabetic retinopathy, Platelet, Neutrophil, NETs

Immuno-engineering and Cellular Therapies

Th142. Chemokine-engineered CAR-T Cells for Autoimmunity: Multiplexed Mrna Technology for Improved Mode of Action and Safety

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Abstract Text: Chimeric antigen receptor (CAR)-T cells have recently emerged as a potential cure for systemic autoimmune diseases but their widespread application in rheumatology is limited by costly manufacturing, poor control over biodistribution and pharmacodynamics and fear of long-term side effects. As the immune reset in autoimmunity does not appear to require long-term CAR-T engraftment, mRNA technology bears the promise for transient CAR-T cell treatments with an improved safety and treatment controllability. Here, we optimized mRNA delivery to primary human T cells using lipid nanoparticles (LNPs) versus electroporation. We developed CAR-T cells co-expressing mRNA-based CD19-CAR or stable CRISPR/Cas9 CAR knock-in (KI) alongside mRNA-encoded chemokine receptors for deeper B cell depletion in lymphatic tissues (CCR7) and transient targeting of inflammation (CXCR3A). Both mRNA transfection methods resulted in effective transfection (>80%) and high T cell viability (>90%). In CRISPR/Cas9-engineered CAR-T cells, CAR-KI rate was >20% and cells could be re-transfected with mRNA 6 days post-KI without significant viability loss. In "mRNA-only" CAR-T cells, co-expression of CAR and chemokine receptor mRNAs could be achieved in 80% of cells upon single treatment and lasted ~3d, whereafter cells assumed their original phenotype. Transfection with CCR7-mRNA strongly boosted the migration of (CAR-)T cells toward CCL21, a marker of secondary lymphoid organs, while CXCR3A-mRNA increased migration toward the inflammatory mediator CXCL11. Chemokine-engineered CAR-T cells outperformed T cells transfected with CAR only in a combined killing and migration *in vitro* assay, demonstrating a functional benefit of multiplexed CAR-T cells. Validation in relevant disease models and transition to GMP-compatible manufacturing are ongoing.

Th143. Engineering of Regulatory T Cells with Hepatocyte-specific Chimeric Antigen Receptor Enhances Liver Tissue Trafficking

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Abstract Text: Regulatory T cells (Tregs), known for their powerful immunomodulatory properties, are being evaluated as a therapeutic strategy to ameliorate immunopathology and re-establish immunological tolerance in autoimmunity and transplantation. Tregs require T cell receptor (TCR)-mediated cognate antigen engagement for activation and pre-clinical studies demonstrated that antigen-specific Tregs preferentially migrate to inflammation sites, enabling targeted immunosuppression. Antigen specificity of Tregs can be modified using synthetic chimeric antigen receptor (CAR) technology. We hypothesized that engineering Tregs to express a CAR targeting a hepatocyte-specific antigen would enhance their selective migration to the liver. To test this, we designed and

synthesized multiple second-generation CAR constructs incorporating single-chain antibody fragments specific for asialoglycoprotein receptor 1 (ASGPR) and varying transmembrane and hinge domains. Initial screening for specificity and functionality was performed using a Jurkat NFAT-reporter cell line stimulated with recombinant proteins and/or ASGPR-expressing cells. Human $CD4^+CD25^+CD127^-$ Tregs were then transduced, using lentiviral vectors, and their specificity and functionality was assessed through activation, proliferation, and suppression *in vitro* assays. The tissue homing and persistence of liver-specific CAR-Tregs were evaluated *in vivo* in immunocompromised NSG mice under homeostatic conditions. CARs were selected based on their specificity for ASGPR and activation profile following antigen recognition. As compared to untransduced polyclonal Tregs, ASGPR-specific CAR-Tregs exhibited enhanced activation, proliferation and suppressive function *in vitro*. Following infusion into NSG mice, CAR-Tregs preferentially homed and persisted in the liver. These findings demonstrate that organ-specific CAR engineering enhances Treg recruitment to target tissues, offering a promising strategy for improving outcomes in transplantation and inflammatory liver diseases.

Th144. Engineering Thymic Selection to Promote Acceptance of Allogeneic Transplantation

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Abstract Text: Achieving donor-specific immune tolerance is critical for graft survival without life-long immunosuppression. Thymic epithelial cells (TEC) are pivotal in central tolerance, selecting a self-tolerant T cell repertoire. We investigated whether mir142-regulated lentiviral vector (LV)-driven expression of donor-related major histocompatibility complexes (MHC) by recipient TEC generates a “hybrid thymus” capable of reshaping T cell repertoire, promoting tolerance to donor tissue transplantation. We first demonstrated that intra-thymic (it)-LV-driven MHC expression in MHC knock-out (KO) mice restored the T cell selection and determined responsiveness to allo-antigens(Ags) of the reconstituted T cell population. Therefore, it-LV injection enables MHC expression on cortical and medullary TEC, restoring positive and negative selection of thymocytes in MHC KO mice. In immunocompetent recipient Balb/c (H-2d), we removed pre-existing T cell repertoire by applying a lymphodepletion regimen before it-LV injection of a mix of LV encoding for donor H-2b molecules to obtain the hybrid thymus (H-2db). The transgenic H-2b MHC expression was confirmed by immunostaining by TEC of the recipient Balb/c. Proliferative response to allo-H-2b of the reconstituted T cells isolated from it-LV injected mice evaluated by mixed leukocyte reaction (MLR) assay showed an alloreactivity re-shaped by donor- H-2b MHC expressed in the thymus. These findings demonstrate the potential of it-LV-mediated MHC gene transfer to promote donor-specific central tolerance.

Th145. Enhancing Adoptive Immunotherapy: Targeting Epstein-Barr Virus with Highly Active, Durable and Immunosuppression-resistant Cytotoxic $CD4^+$T Cells

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Abstract Text: Solid organ transplantation (SOT) requires prolonged/life-long immunosuppression, often causing Epstein-Barr Virus (EBV) reactivation and post-transplant lymphoproliferative disease (EBV-PTLD). Adoptive transfer of EBV-specific cytotoxic T cells (EBV-CTLs) may rapidly restore immune competency and control EBV-PTLD. However, clinical efficacy is often limited by exhaustion of *in vitro* expanded CTLs (mostly $CD8^+$) and/or by ongoing immunosuppression. Here, we hypothesized that EBV-specific Th1/17-like $CD4^+$-CTLs programmed to maintain high potency, self-renewal, and resistance to immunosuppression could be a superior therapeutic strategy. *Ex vivo* generated EBV-specific $CD4^+$-CTLs (Th1/17-CTLs) demonstrated superior recognition and polyfunctional responses, concurrently secreting TNF-α, IFN-γ, IL-2, and inducible Granzyme B against EBV-antigens compared to the standard Th1-CTLs. Th1/17-CTLs displayed less differentiated central memory (TCM) ($CCR7^{hi}$, $CD27^{hi}$) and

resident memory (TRM) ($CD103^{hi}$, $CD69^{hi}$) phenotype, unlike the predominantly effector memory (TEM) ($CCR7_{low}$, $CD103_{low}$) Th1-CTLs. Paradoxically, a second stimulation further enhanced Th1/17-CTLs' polyfunctionality, robust proliferative capacity, superior bioenergetic/mitochondrial stability, and spare respiratory capacity, unlike the Th1-CTLs. Importantly, Th1/17-CTLs overexpressed Multi-Drug Resistance Protein 1 (MDR1) and displayed resistance to tacrolimus-mediated immunosuppression. Functionally, Th1/17-CTLs exhibited superior recognition and cytotoxicity against autologous EBV lymphoblastoid cell lines (EBV-LCLs). Adoptive transfer of Th1/17-CTLs led to the eradication of systemic PTLD-like disease in humanized mice, demonstrating *in vivo* persistence and superior self-renewal ability without cytokine support. This study introduces a novel, highly functional, non-exhausted oncoprotein-specific T cells with superior cytotoxic capability and resistance to immunosuppression, addressing key limitations of existing T cell therapies in immunocompromised patients, paving the way for more robust immune effectors targeting other cancer antigens in solid and hematological malignancies.

Th146. Pooled CRISPR Screening for Drug Target Discovery in Primary Human Myeloid Cells

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Abstract Text: CRISPR screens have become the core discovery engine in modern biology and are widely used to uncover novel targets for immunotherapy. These genetic screens are usually performed in simplistic cell models and often coupled to readouts such as cell fitness. In contrast, Perturb-Seq or CROP-Seq screens combine CRISPR perturbations with single-cell transcriptomics, enabling information-rich phenotyping in physiologically relevant primary cell types. Thereby, cells are typically transduced with lentiviruses comprising a pooled sgRNA library and a Cas effector protein followed by measuring transcriptomic profiles of individual cells. Macrophages are pivotal in the maintenance of tissue homeostasis and have been directly linked to a wide range of diseases, including infection, inflammation and cancer. This renders them attractive therapeutic targets, but their specific functions in pathogenesis often remain elusive. High-content CRISPR screens can help dissecting their diverse roles, yet the genetic manipulation of primary human macrophages – and especially the delivery of the CRISPR machinery by lentiviruses – has proven extremely challenging. At Myllia, we have built a technology platform for CROP-Seq screens in primary human macrophages, which were previously inaccessible for genetic perturbation screens at scale. We developed a first-of-its-kind CROP-CITE-Seq screening workflow to assess macrophage identity as well as activation state by measuring transcriptomic and proteomic changes upon pooled perturbation of more than 50 target genes. Our results recapitulate the role of well-established mediators of immune signaling in macrophages. Ultimately, genetic screens in primary cells that reflect human disease biology will lay the foundations for successful drug discovery campaigns

Th148. Rapid Preclinical Development of Immtac Bispecifics in Mammalian Protein Expression Systems

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Abstract Text: ImmTACs are a novel class of fusion proteins combining a CD8-associated TCR domain with the immune-activating properties of an anti-CD3ζ single-chain variable fragment (scFv). These molecules trigger anti-tumor responses by targeting peptide-loaded HLA complexes presented by cancer cells. Tebentafusp, a gp100(280-288)/A2-specific ImmTAC, has shown superior overall survival in uveal melanoma compared to anti-PD1 therapy. Tebentafusp is produced using recombinant DNA technology in E. coli, a system that limits the proper folding and activity of complex eukaryotic proteins. Here, we describe the use of a serum-free mammalian system for ImmTAC production and the functional characterization of Tebentafusp and IMDA-001, a modified version with a proprietary anti-CD3ζ scFv. Chinese Hamster Ovary cells (CHO-S) transduced with lentiviral vectors encoding the ImmTAC

cDNA were cultured in fed-batch conditions for protein production for up to 14 days. ImmTACs were purified from culture supernatants with affinity columns and, after final formulation, yielded 168 mg/L for Tebentafusp and 126 mg/L for IMDA-001. Functionality was validated by cytotoxicity assays, where both proteins exhibited potent T cell-mediated killing of gp100(280-288)/A2^{+} target cells at picomolar concentrations. IMDA-001 demonstrated enhanced cytotoxicity, higher proinflammatory cytokine secretion, and preferential activation of CD4^{+} T cells compared to Tebentafusp. This study provides a robust and scalable method for bi-specific protein production in CHO-S cells and accelerated ImmTAC pre-clinical development from conceptualization to functional testing within only two months. These findings support the use of mammalian systems for the production of next-generation ImmTACs and underscore the potential for further optimization, as exemplified by IMDA-001.

Th149. Single Cell protein Interactomics using Proximity Networks Help Unravel Membrane Protein Architecture of CD19 CAR T Cells at Rest and During Tumor Interaction

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Abstract Text: Diseases are caused by aberrant cell function. These functions are executed by proteins interacting with other proteins and clustering. This dynamic interactome represents a vast and unstudied dimension in translational research with massive potential as novel biomarkers, drug targets, and discoveries of new therapeutic avenues. Protein interactomics by Proximity Networks delivers this new exciting omics dimension. Herein, we present the Proximity Network Assay, where 155 target proteins are positioned in a DNA-based spatial network of >40,000 nodes from single cells without optics. Barcoded antibodies bound to target proteins are subjected to in situ rolling circle amplification, generating multiple barcode copies that subsequently serve as spatial network nodes to several other proximal neighbors via the hybridization of a linker-oligonucleotide. Pairs of proximal barcodes are inserted into the two ends of the linker by gap-fill ligation, readable by DNA sequencing. We utilized the Proximity Network Assay to comprehensively analyze the membrane protein organization of CD19 CAR T cells in both resting condition and during coculture with CD19-expressing tumor cells. Proximity Network Assay provided critical insights into the nano-organization and functional dynamics of CAR T cell membranes, demonstrating the utility of the Proximity Network Assay in uncovering the mechanistic underpinnings of CAR T cell biology. This knowledge paves the way for optimizing CAR T cell therapies to enhance their efficacy and persistence in clinical settings. At nanoscale resolution in 3D, Proximity Networks will be applicable to high throughput protein interactomics studies in immuno oncology, hematology, and cell therapy research.

Th150. Single-Cell Analysis of TCR and Gene Expression Diversity in NY-ESO-1-Specific HLA-DRB3*02:02-Restricted CD4^{+} T Cells from Healthy Donors

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Abstract Text: Antigen-specific natural transgenic T cell receptors (tTCRs) have been successfully isolated against NY-ESO-1 119−143, a cancer/testis antigen with minimal expression in healthy tissues but broad presence in tumors, making it a promising immunotherapy target. Unlike CAR T cell therapies, which recognize only extracellular proteins, tTCRs enable the recognition of peptides from both intracellular and extracellular sources, expanding their therapeutic potential. However, most TCR-based therapies target HLA class I alleles, which are susceptible to downregulation, allowing tumors to evade immune detection. To overcome this, we focused on the HLA-DRB3*02:02 allele, expressed in ~50% of the Caucasian population. CD4^{+} T cells were pre-sensitized and expanded *in vitro*

using NY-ESO-1 119−143-pulsed monocytes. $CD4^+$ $CD154^+$ T cells were sorted and expanded through multiple rounds to enhance specificity. After 30 days, a highly specific $CD4^+$ CD25− T cell population targeting NY-ESO-1 119−143 on HLA-DRB3*02:02 was obtained, maintaining 97% CD4 purity, with specificity significantly improving after the second sorting round. Single-cell RNA sequencing (scRNAseq) and TCR sequencing (scTCRseq) were performed to analyze TCR repertoires, gene expression, and clonal expansion. This analysis identified potential TCR candidates for therapeutic development. These findings demonstrate the feasibility of isolating highly specific $CD4^+$ TCRs against NY-ESO-1 119−143 in the context of HLA-DRB3*02:02, supporting the potential of this strategy as an effective immunotherapy for NY-ESO-1-expressing tumors.

Tu153. Treatment of a Humanized GVHD Model with PanTreg is Clinically Superior to Adalimumab and Induces Infectious Immune Tolerization of the Systemic Immunome

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Abstract Text: PanTreg design: The antigen binding domain is directed towards TNF-alpha. This enables homing to the diseased microenvironment of any autoimmune disease in which TNF-alpha is expressed and drives pathogenesis, including Rheumatoid Arthritis, Psoriasis, IBD, and also GVHD. The construct constitutively expresses FoxP3, to confer and stabilise a Treg functional phenotype. Tolerogenic cytokines IL-10 and TGF-beta are induced upon TNF binding. Thus, upon activation, PanTreg cells target a main pathogenic pathway, without depending on a purported etiologic antigen. Inducible cytokine production also reduces risks of off-target effects. PanTreg can target virtually any disease where TNF is pathogenically relevant. We validated this approach in a humanised model of GVHD, leading to a complete control of the disease with highly significant differences in clinical outcomes when compared with state of art therapies such as anti-TNF (Adalimumab), or polyclonal Tregs. We compared the changes induced by PanTreg on the architecture of the Immunome systemically with Adalimumab treatment, leveraging an AI-powered single cell proteomics platform. PanTreg treatment determined a significant decline in inflammatory Teff and an increase in the production of tolerogenic cytokines, all elements associated with the induction of infectious immune tolerance. Conversely, treatment with Adalimumab did not reduce inflammatory memory Teff, possibly attributing its inferior clinical efficacy with a simple inhibition of a circulating cytokine, without affecting, unlike PanTreg, the underlying cellular mechanisms of autoimmunity. Spacial transcriptomics data showed a preservation of tissue architecture and minimal inflammatory infiltration in PanTreg-treatment, as opposed to Adalimumab, where tissue destruction was accompanied by inflammatory Teff infiltration.

Tu154. Use of Engineered CAR-Treg Cells to Induce Immune Tolerance in Liver Transplantation – Insights from the LIBERATE Clinical Trial

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Abstract Text: LIBERATE is a first-in-human Phase I/IIa clinical trial designed to evaluate the safety and activity of autologous engineered Tregs in promoting liver transplantation tolerance in HLA-A2-negative patients who have received a graft from an HLA-A2-positive donor. A proprietary GMP manufacturing process has been developed to engineer recipient-derived QEL-001 cell products that express an anti-HLA-A2 targeted CAR, with a FOXP3 phenotype-lock, and a safety-switch. An extensive characterization of QEL-001 CAR-Treg products has

demonstrated the maintenance of phenotypic and functional characteristics of unmodified-Tregs. A safety cohort consisting of three patients found QEL-001 to be well tolerated within the protocol-defined dose range, with no serious adverse events or dose-limiting toxicities. Comprehensive immunomonitoring of blood samples by FACS and scRNAseq analysis showed persistence of CAR-Tregs in circulation for up to 12-months post-infusion, and maintenance of canonical markers of stable and effective Tregs. Furthermore, liver biopsies collected post-infusion demonstrated graft trafficking and phenotypic stability, leading to substantial enrichment of CAR-Tregs in the liver compared to the periphery. An expansion cohort is currently being conducted with the addition of lymphodepletion by low-dose rATG previous to QEL-001 infusion. Active ATG was cleared from circulation prior cell infusion and mediated a significant reduction in T cell frequencies. QEL-001 CAR-Treg engraftment levels in circulation was improved compared to the safety cohort who did not receive rATG-conditioning, and the Treg/Teff ratio increased favoring immunoregulation. This study provides pioneering data on the homeostasis and functionality of engineered Tregs, informing the development of future tolerogenic therapies in a broad range of clinical settings.

Tu155. Utilizing Phage Display to Select Polypeptides with Affinity to CD8

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Abstract Text: Phage display is a powerful protein engineering technique that utilizes the diversity of a bacteriophage library to select polypeptides with specific binding properties. The library is composed of phages that are engineered to display different polypeptide sequences on the surface of their coat proteins. Phage display involves several rounds of selection in which the phage library is incubated with a target protein immobilized on a solid surface. Phages that bind to the target protein undergo affinity purification and amplification. After several rounds of selection, the phage genome is sequenced to identify polypeptide sequences that bind with high affinity to the target. Phage display has many applications, including developing therapeutics, studying protein-protein interactions, and discovering antibody epitopes. We are using a phage display library expressing dodecamer polypeptides to discover a polypeptide sequence that binds to the CD8 alpha protein, found primarily on the surface of cytotoxic T cells. This sequence could be used to recruit $CD8^+$ T cells for targeted cell killing, such as for killing cancer cells. We predict that phage display can also be utilized to discover the epitope bound by OKT8, a unique anti-CD8 antibody that stimulates T cell activation. This information will be useful in developing immunotherapeutics by employing a CD8-targeted polypeptide to identify and recruit T cells and elucidating a stimulatory T cell epitope on CD8.

Tu156. Versatile and Efficient Integration of Large Therapeutic Transgenes in Human T Cells via Nuclease-mediated Editing and Serine Integrases

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Abstract Text: Adoptive T cell therapy using gene-modified cells expressing target-specific receptors has emerged as a powerful treatment modality for malignancies and autoimmune diseases. However, current gene transfer methods, such as lentiviral transduction and homology-directed DNA repair (HDR), are limited in their ability to integrate large DNA sequences, restricting our potential to engineer T cells. To address these challenges, we developed PASTA (programmable and site-specific transgene addition), a method enabling efficient, site-specific integration of large transgene cassettes. PASTA functions in two phases: Phase 1 integrates a small DNA landing pad into the genome using CRISPR-Cas-mediated HDR. Phase 2 employs a large serine integrase (e.g., Bxb1) to insert a circular DNA vector at the landing pad. This “one-pot” process is initiated by a single transfection, simplifying

the procedure and making it suitable for clinical application. We demonstrate that 'one-pot' PASTA allows efficient transgene integration at multiple sites relevant for T cell engineering (TRAC, B2M, CD3E). The integrase-mediated strategy outperforms conventional HDR by 9-fold (range: 2.5-14-fold) when introducing large transgenes exceeding 6.5 kilobases, and achieving integration rates of >20% of transfected T cells. Consequently, 'one-pot' PASTA enables the precise incorporation of multicistronic constructs and co-expression of multiple antigen receptors and a safety switch. Resulting multi-antigen targeting CAR T cell are currently under evaluation in B cell lymphoma models. Overall, 'one-pot' PASTA is a versatile and efficient non-viral gene transfer platform for enhanced T cell engineering, offering a simpler and more flexible alternative to prime editing-based approaches like PASSIGE and PASTE.

Tu157. A Scalable and Robust Method to Produce and Obtain Pure Lentiviral Vectors for Point-of-care CART-cell Therapy Developed in Academic Settings

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Abstract Text: The Hospital Clinic de Barcelona (HCB) is a European leader in developing CART-cells targeting CD19 for B cell malignancies. Indeed, its (Varnimcabtagene autoleucel, Var-cel) therapy for relapsed/refractory acute lymphoblastic leukemia has been approved for hospital exemption use and received PRIME designation by the EMA. Var-cel, as other CAR-T therapies, relies on lentiviral vectors (LVVs) to deliver transgenes to autologous T cells. Thus, optimizing and scaling LVVs production is crucial to meet growing demand and regulatory standards. We developed an optimized and scalable LVV production pipeline at HCB using a fixed-bed bioreactor (Scale-X Hydro) achieving a threefold increase in LVV productivity compared to our conventional, currently-approved, upstream production (USP) using 2D multi-layer flasks (~$6x10^6$ versus ~$2x10^6$ TU/cm²). The revised USP was based on prior optimizing of 293T cell growth and transfection conditions. Further downstream processing (DSP) was also refined to yield high LVV purity with minimal loss of functional titer. DSP steps included DNAse treatment, clarification via filtration, concentration/diafiltration using hollow fiber filters and fast protein liquid chromatography with multimodal resin, which enabled the removal of over 99% of process-related impurities while retaining a high LVV functional titer (~$2.5x10^8$ TU/ml) and a recovery rate exceeding 60%. Our optimized LVVs production and purification pipeline aligns with industry standards and supports efforts to secure EMA marketing authorization, ensuring scalable LVV supply for point-of-care CAR-T therapies.

Tu158. Affinity-tuning of Human anti-HLA-A2 Chimeric Antigen Receptors Enhances Their Function

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Abstract Text: Regulatory T cells (Tregs), known for their potent immunomodulatory properties, can be genetically engineered using chimeric antigen receptor (CAR) technology to target organ-specific antigens. CARs exhibit supraphysiological affinity to their target antigens compared to T cell receptors. However, the effect of modifying the CAR's single-chain variable fragment (scFv) affinity on CAR-Treg functionality remains unclear. In this study, we aimed to evaluate the functional properties of affinity-tuned human anti-HLA-A2 CAR-Tregs. Second-generation anti-HLA-A2 CAR constructs expressing low-affinity (LA) and high-affinity (HA) scFvs were designed. Human HLA-A2 negative $CD4^+CD25^+CD127^-$ Tregs were transduced using lentiviral vectors. The antigen-specific activation and suppressive function of CAR-Tregs were assessed *in vitro* using cell lines and precision-cut liver slices (PCLS). Therapeutic efficacy was evaluated in an *in vivo* xeno graft-versus-host disease (GvHD) by infusing CAR-Tregs

together with HLA-A2+ PBMCs into irradiated NSG mice. Mechanistic studies focused on CAR avidity, internalization and recycling using dextramer tracking. When cultured with HLA-A2 expressing cells, LA CAR-Tregs exhibited the highest proportion of activated alloantigen-specific cells and suppressive effects. Furthermore, when cultured with PCLS, they showed greater tissue infiltration than other cells. *In vivo*, LA CAR-Tregs exhibited improved persistence, delayed GvHD onset, and prolonged recipient survival. Mechanistically, HA CAR-Tregs exhibited higher avidity for their target antigen but underwent rapid CAR internalization, and reduced dextramer expression upon exposure to physiological antigen levels, indicating CAR downregulation. Affinity-tuning the CAR of anti-HLA-A2 CAR-Tregs significantly alters their functionality, both *in vitro* and *in vivo*, which has implications for the design of engineered Treg therapies for clinical use.

Tu159. Armoured CAR Tregs with PD1 Promoter-driven IL-10 Have Enhanced Suppressive Function

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Abstract Text: Adoptive transfer of regulatory T cells (Tregs) is currently under clinical investigation to treat autoimmunity and transplant rejection. Clinical trials have confirmed the safety and feasibility of this approach, and mouse studies have shown that Treg therapy has increased efficacy when cells are given disease-relevant specificity via expression of chimeric antigen receptors (CARs). However, CAR-Tregs have yet to induce long-lasting tolerance in mice, thus suggesting the need for additional cell engineering. To this end, we aimed to engineer CAR-Tregs to remove PD1, a negative regulator of Treg activation, and introduce IL-10 production under control of the PDCD1 (PD1) promoter. Human CAR-Tregs were generated through lentiviral transduction with an HLA-A2-specific CAR. Homology directed repair at the PDCD1 locus was subsequently used to knock-out PDCD1 and introduce IL10 and a constitutively-expressed, truncated CD19 (tCD19) reporter. Following transduction, CAR-Tregs maintained high expression of key Treg markers, including FOXP3 and Helios. PD1 deletion enhanced CAR-stimulated Treg activation potential. Knock-in of IL10 into the PDCD1 locus resulted in CAR-stimulated expression of IL-10 at levels exceeding those produced by type 1 regulatory (Tr1) cells. RNA sequencing data showed that Treg identity remained intact and distinct from Tr1 cells. IL-10-expressing CAR-Tregs also had improved suppressive function, with superior control of dendritic cells as well as alloantigen- and islet autoantigen-specific T cell activity. When tested in a humanized mouse model of graft versus host disease, IL-10-expressing CAR-Tregs were stable and potently suppressive. Together, these data show that IL-10-armoured CAR-Tregs could enhance the clinical efficacy of Treg therapies.

Tu160. Chimeric Antigen Receptor Induced Signaling Imbalance Compromises Human Regulatory T Cell Function

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Abstract Text: Regulatory T cells (Tregs) hold tremendous promise as living therapeutics against autoimmunity and transplant rejection. Antigen specificity is key for Treg efficacy. Yet, while chimeric antigen receptor (CAR) engineering enables new antigen specificities, CAR Tregs have shown limited efficacy in solid organ transplant rejection and autoimmune disease models, even when combined with immunosuppressive regimens. In contrast, antigen-specific T cell receptor (TCR) transgenic Tregs alone reverse autoimmune disease. We hypothesized that CAR signaling fails to fully recapitulate TCR/CD28 signaling in Tregs. Single-cell RNA-seq of Tregs and effector T

(Teff) cells activated via CAR or TCR/CD28 identified a pro-inflammatory FOXP3$^+$HELIOS$^+$ Treg subset uniquely induced by CAR signaling. These cells were characterized by high expression of inflammatory cytokines and chemokines, such as IFNy, granzyme B, and CD40L at the mRNA and protein levels. Gene pathway analysis revealed that CAR, but not TCR/CD28, uniquely induces noncanonical NF-kB pathway activity in Tregs to levels comparable to those in activated Teff cells. Consistently, NF-kB inhibition reduced IFNy by CAR Tregs. Interestingly, reducing CAR affinity by 40-fold rescued noncanonical NF-kB pathway activity to the level found in TCR/CD28 activated Tregs. Transcription factor enrichment analysis revealed reduced BATF expression specifically in high affinity CAR Tregs. Ectopically expressing BATF in CAR Tregs curbed IFNy production and enhanced suppressive function. These findings shed light on key signaling pathways controlling CAR Treg biology. Ongoing studies are dissecting the impact of NF-kB and BATF on CAR Treg function in humanized mice towards designing the next generation of CAR Treg therapies.

Tu161. Chimeric Anti-HLA Antibody Receptor Engineered Regulatory T Cells (CHAR Tregs) Suppress Alloantigen-specific B Cells from Pre-sensitized Transplant Recipients

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Abstract Text: Organ transplantation is a lifesaving procedure, with 50,000 transplants happening every year in the United States. However, many patients harbor antibodies and B cells directed against allogeneic human leukocyte antigen (HLA) molecules, notably HLA-A2, greatly decreasing their likelihood of receiving a compatible organ. Moreover, antibody-mediated rejection is a significant contributor to chronic transplant rejection. Current strategies to desensitize patients non-specifically target circulating antibodies and B cells, resulting in poor efficacy and complications. Regulatory T cells (Tregs) are immune cells dedicated to suppressing specific immune responses by interacting with both innate and adaptive immune cells. Here, we genetically modified human Tregs with a chimeric anti-HLA antibody receptor (CHAR) consisting of an extracellular HLA-A2 protein fused to a CD28-CD3zeta intracellular signaling domain, driving Treg activation upon recognition of anti-HLA-A2 antibodies on the surface of alloreactive B cells. We find that HLA-A2 CHAR Tregs get activated specifically by anti-HLA-A2 antibody-producing cells. Of note, HLA-A2 CHAR activation does not negatively affect Treg stability, as measured by expression of the Treg lineage transcription factors FOXP3 and HELIOS. Interestingly, HLA-A2 CHAR Tregs are not cytotoxic towards anti-HLA-A2 antibody-producing cells, unlike HLA-A2 CHAR modified conventional CD4$^+$ T cells. Importantly, HLA-A2 CHAR Tregs recognize and significantly suppress high affinity IgG antibody production by B cells from HLA-A2 sensitized patients. Altogether, our results provide proof-of-concept of a new strategy to specifically inhibit alloreactive B cells and support designing CHAR Tregs displaying different HLA class I and class II molecules on their surface as targeted therapeutics to desensitize transplant recipients.

Tu162. Clinical Quantification of Cardiovascular Disease Marker C-reactive Protein Using Radioimmunoassay and ELISA Immunoassay

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Abstract Text: C-reactive protein (CRP) is an acute-phase protein synthesized in the liver, an inflammatory marker. CRP levels in the blood reflect acute or chronic inflammation; recently, CRP has become a potent cardiovascular marker. However, detection methods are not sensitive enough. Hence, sensitive radioimmunoassay and direct ELISA for CRP have been developed. We optimized two sets of standards for CRP radioimmunoassay: (1) a high standard range (0-6400 ng/mL), which can measure up to 1,60,000 ng/mL. (2) a highly sensitive assay (0-200 ng/mL), which can measure the CRP level in human serum as low as 3 ng/mL. CRP was radiolabelled using the

chloramine-T method. A magnetizable cellulose particle-based separation system was adopted, which is user-friendly with minimum pipetting steps and an incubation time of 1 h at room temperature. The anti-CRP antibody was conjugated with HRP using the sodium periodate oxidation method for direct ELISA. Optimized assay protocol: Add 100 µL standards (0 to 100 ng/mL) to each well and incubate for 2 hrs at RT, then the plates were washed. The blocking was carried out for 30 minutes, followed by washing. Then, add 100 µL of anti-CRP-pAb-HRP to each well and incubate for 1 hr. Afterwards, 50 µL TMB was added to wells, 0.2M H2SO4 was added to stop the reaction, and the absorbance was measured at 450 nm to plot the standard graph. Optimized assays were analyzed and validated for assay sensitivity, precision, dilution linearity, recovery, and cross-reactivity. The assay covers the clinical range of human CRP analysis for clinical tests.

Tu163. Cytokine-driven Modulation of CART84 Phenotype and Function for AML Therapy

Manel Juan[1], Maria Val-Casals[1], Lorena Pérez-Amill[2], Mercedes Armand-Ugón[2], Ane Altuna[1], Sergio Peña[2] and Nela Klein-González[1]

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Abstract Text: CD84 antigen is an immunoreceptor overexpressed in acute myeloid leukemia (AML). We developed a Chimeric Antigen Receptor (CAR) T cell targeting CD84 (CART84), which has demonstrated efficacy against AML in preclinical models. Less differentiated T cell subsets within CAR T cell products are associated with improved persistence and potency. Interleukin (IL) 21 promotes the expansion of the long-lived memory stem T cell (TSCM) compartment, which enhances CAR T cell proliferation and antitumor activity. This study aimed to characterize the phenotype and functionality of CART84 cells expanded with different cytokine combinations. Healthy donor T cells were transduced with a lentiviral vector encoding an anti-CD84 CAR (antiCD84scFv-CD8αTM-4-1BB-CD3z) and expanded in three conditions: IL-2; IL-7 and IL-15 (IL7$^+$15); and IL-7, IL-15 and IL-21 (IL7$^+$15$^+$21). We then evaluated their memory phenotype, metabolic capacity, and cytotoxicity towards the CD84$^+$ AML cell line MOLM-13. We did not observe any differences in the TSCM compartment across conditions. However, the IL-7$^+$15$^+$21 condition yielded the highest proportion of central memory (TCM) CD4 cells, correlating with reduced differentiation into effector memory (TEM) and terminally differentiated effector (TTE) subsets, alongside increased ATP production. Regarding efficacy, both CART84 expanded under IL-7$^+$15 and IL-7$^+$15$^+$21 conditions displayed greater cytotoxicity towards MOLM-13 cells *in vitro* compared to the IL-2 condition. In conclusion, although CART84 expansion with IL-21 addition did not increase the TSCM compartment, it promoted a central memory-enriched phenotype in CART84 cells , enhancing metabolic activity and cytotoxic efficacy against CD84$^+$ cells. These results further support the potential of IL-21 in optimizing CAR T cell therapies for AML treatment.

Tu164. Development of Allogeneic Cmv-virus-specific CAR T Cells Targeting CD84 for Acute Myeloid Leukemia

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Abstract Text: Allogeneic virus-specific T cells (VSTs) are a promising platform for off-the-shelf cell therapies due to their restricted T cell receptor (TCR) repertoire, which has enabled their safe application as antiviral clinical therapies. CD84 is a surface antigen highly expressed on acute myeloid leukemia (AML), and CD84-targeted chimeric antigen receptor (CAR) T cells have demonstrated strong cytotoxic activity against AML in preclinical models. In this study, we engineered cytomegalovirus (CMV)-specific VSTs (CMV-VSTs) to express a CAR targeting

CD84. The resulting CAR84-CMV-VSTs could not only target CD84 on AML through the CAR, but also recognize CMV antigens via their endogenous CMV-restricted TCR repertoire. Thus, we propose CAR84-CMV-VSTs as a potential off-the-shelf CART-cell therapy for the treatment of relapsed/refractory (R/R) AML. We successfully expanded CMV-VSTs from healthy donor peripheral blood mononuclear cells (PBMCs) using CMV pp65 peptides. These VSTs demonstrated remarkable CMV specificity, with an average of 33% of $CD4^+$ and 38% of $CD8^+$ T cells responding to pp65 peptides, as shown by CD107a and IFNγ staining. Furthermore, CMV-VSTs exhibited robust functional activity, selectively lysing both autologous and allogeneic phytohemagglutinin-induced blasts presenting pp65 peptides. Subsequently, CAR84-CMV-VSTs were generated by transducing CMV-VSTs with a lentiviral vector encoding a CD84-directed second-generation CAR (antiCD84scFv-CD8αTM-4-1BB-CD3z). CAR84-CMV-VSTs exhibited potent cytotoxicity against $CD84^+$ MOLM-13 cells (an AML cell line) *in vitro*, underscoring their therapeutic potential. These findings support CMV-VSTs as a platform for developing allogeneic CAR T cell therapies. Optimizing transduction and expansion protocols will be critical to enable scalable, off-the-shelf production of CAR84-CMV-VSTs for clinical applications in R/R AML.

W104. Gut Microbiota and Metabolites Influence CAR-T Cell Persistence, Efficacy and Predict Outcomes in Multiple Myeloma

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Abstract Text: Multiple myeloma is a hematologic malignancy that remains incurable despite advances in immunotherapies like CAR-T cell therapy. This study investigates the role of gut microbiota in clinical outcomes for multiple myeloma patients treated with the humanized BCMA-directed CAR-T therapy, ARI0002h. Gut microbial metabolites, particularly succinate, were associated with CAR-T cell phenotypes and persistence. In CAR-T cell cultures, higher succinate levels correlated positively with $CD4^+$ central memory cells, enhancing respiratory capacity. In a murine myeloma model, a diet increasing intestinal succinate levels improved CAR-T cell persistence and tumor control. Furthermore, specific bacterial taxa like Acidaminococcaceae, Monoglobaceae, or Akkermansiaceae and metabolites like tyrosine associated with CAR-T cell clinical outcomes. These microbial profiles were integrated into predictive response models that identified patients likely to achieve a complete response by day 100 and 180 post-infusion. Additionally, a response improvement model effectively identified patients likely to improve their response from a partial to a complete response by day 100 post-infusion These findings suggest that gut microbiota correlates with CAR-T cell therapy efficacy and can be a valuable tool for risk prediction.

W168. Engineering NK Cells for Metabolic Resilience in the Tumor Microenvironment of Glioblastoma Multiforme

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Abstract Text: Glioblastoma multiforme (GBM) remains an incurable form of brain cancer with only 5-6% of people diagnosed surviving beyond 5 years, a statistic unchanged since 2005. Recent advancements in cellular immunotherapy present new treatment opportunities for GBM, and emerging evidence indicates that natural killer (NK) cells can kill both mature GBM tumor cells and associated tumor stem cells *in vitro*. However, despite their relatively high presence within GBM tumors, NK cells lose their cytotoxic potential in the tumor microenvironment (TME). The TME of solid tumors is characterized by nutrient depletion, hypoxia, and excess of immunosuppressive metabolites and lipids, all contributing to NK cell dysfunction. Therefore, optimizing the metabolic function of NK cells to maintain and enhance their cytotoxicity within the GBM TME is crucial for the success of NK cell therapies. We have recently developed a cellular platform of adaptive single-KIR^+NKG2C^+ NK cells which exhibit potent anti-tumor activity, which will be further enhanced through genetic engineering of various metabolic targets to improve metabolic resilience. Methods for testing and characterization of these genetically engineered NK cells include the use of GBM cell lines and primary human glioblastoma cell lines (brain tumor initiating cell lines/BTICs) in 3D multicellular tumor spheroid models, along with functional profiling of metabolism and nutrient uptake. This research strives to develop novel therapeutic approaches to strengthen therapeutic NK cells, paving the way for effective NK cell-based immunotherapies for GBM.

W169. Engineering Relapse-resistant CAR T Cells for Blood Cancers (Penta T Cells)

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Abstract Text: Immunotherapy with chimeric antigen receptor (CAR) T cells has demonstrated remarkable success in treating hematological malignancies. Despite impressive outcomes, relapse driven by antigen-negative disease remains a critical challenge. To overcome tumor antigen escape, effector T cells are engineered to co-express two CARs with distinct antigen specificities such as $CD19^+CD20$ or $CD19^+CD22$. However, tumor relapses still occur and limitations in the manufacturing of multi-receptor CAR T-cells restrict clinical translation, highlighting the need for innovative approaches. We developed a platform for engineering and manufacturing multi-receptor CAR T-cells accommodating transgenes beyond current size limitations. As proof of concept, we generated the first T cell with five independent receptors based on a single-vector design (Penta T cell): four CARs to enable simultaneous targeting of CD19/CD20/CD22/CD79b and mLNGFR for monitoring. *In vitro* studies using single-antigen-positive Ramos tumor models validate that, in contrast to monospecific anti-CD19 CAR T cells, the cytotoxic activity of Penta T cells is not limited to $CD19^+$ cancers but extends to $CD20^+$, $CD22^+$, and $CD79b^+$ tumors. Moreover, by combining single-antigen-positive tumors to create an antigen-heterogeneous cancer model (mixed $CD19^+/CD20^+/CD22^+/CD79b^+$ tumor), we demonstrate that Penta T cells effectively clear heterogeneous cancers by targeting all four antigens simultaneously. In contrast, anti-CD19 CAR T-cells eliminate only the $CD19^+$ tumor fraction, resulting in antigen-escape. Our work represents a crucial proof-of-concept for relapse-resistant CAR T-cells, addressing current barriers in CAR T-cell design and production.

W170. Fate Mapping of Human T Cell Subsets During Expansion for T Cell Therapy

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Abstract Text: Adoptive T cell therapies represent a major advance for patients with otherwise-refractory blood cancers. Yet patient responses can vary widely, due in part to the inherent heterogeneity of the manufactured T cell product. Certain T cell subsets/phenotypes in the infusion product can predict clinical efficacy, but it is unclear how and where these desirable cells arise during therapeutic T cell expansion. We investigated the contribution and fate of multiple T cell subsets (naive, central-memory, effector-memory) expanded alone versus together in a pool towards the final T cell product. We used lentivirus-based proteomic barcodes and mass cytometry to follow these populations over time. When expanded together in a pool, all three subsets displayed a greater degree of terminal differentiation and effector cytokine production than when expanded alone. However, only naive CD8 T cells expanded in the pool showed enhanced cytotoxic potential. Meanwhile, pooled naive and effector-memory T cells, but not central-memory T cells, exhibited greater glycolytic activity and oxidative phosphorylation as measured by single-cell metabolic regulome profiling. This coincided with a restoration of their cell cycle progression by the end of an 11-day expansion, compared to their counterparts expanded alone which gradually returned to G0/G1 over time. Overall, these results suggest that pooled expansion may only be beneficial for certain subsets for T cell therapies.

W171. Gamma Delta T Cells Arming Bispecific Antibodies: Application to Neuroblastoma

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Abstract Text: γδ T cells are alternative cytotoxic effectors that recognize tumors through TCR and innate receptors, with limited lifespan that can be offset with multiple doses from clinical-grade expansion. In this context, bispecific antibodies (BsAb) arming T cells are a viable and safer alternative to CAR transduction. Targeting neuroblastoma through O-acetyl-GD2 (OAcGD2), a GD2 variant with higher cancer-specificity, or B7-H3, a cancer-antigen with a broader expression and immunosuppressive roles, may further enhance efficacy. We aim to propose alternatives to GD2 immunotherapy through production B7-H3 & OAcGD2/Vγ9Vδ2T-cells BsAbs and assessment of their functionality against neuroblastoma. Using the Expi293 system, two BsAbs were produced containing an anti-Vδ2 nanobody linked to anti-OAcGD2 (B6D7) or B7-H3 (376D7) single-chain variable fragment. Purification involved 6xHis-tag binding on cobalt columns. Through flow cytometry, EC50 was assessed for BsAbs characteristics: binding to targets, induction of Vγ9Vδ2T-cells degranulation, and anti-neuroblastoma cytotoxicity at different effector: target (E:T) ratios. Binding of both construct (B6D7 & 376D7) was observed on the $OAcGD2^{+}/B7\text{-}H3^{+}$ LAN-1 cells, but absent on $OAcGD2^{-}/B7\text{-}H3^{-}$ Neuro2a cells. Similarly, equivalent binding on γδT-cells was detected for 376D7 and B6D7. Both constructs were able to induce degranulation of Vγ9Vδ2T-cells against antigen positive (OAcGD2 or B7-H3) cells. Furthermore, both constructs triggered cytotoxicity against antigen positive cells, with the percentage of apoptotic tumoral cells increasing with BsAb dose and E:T ratio In conclusion, δT-cells BsAb can be produced and purified, and are functional. Ongoing work includes assays accounting for recently identified neuroblastoma cell identity diversity and constructs incorporating IL-15.

W172. GNTI-932: Gut-specific CAR-Treg Cells Exhibit Enhanced Localization and Therapeutic Efficacy in Pre-clinical Models of Colitis.

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Abstract Text: Polyclonal Treg cells have demonstrated safety and tolerability in trials for refractory inflammatory bowel disease (IBD). However, limited efficacy has been observed, likely due to challenges in Treg persistence and target specificity. To overcome this shortcoming, we have developed an engineering strategy to generate Treg cells (EngTreg) from human bulk CD4 T cells that constitutively express FOXP3 for stability; contain a rapamycin-activated chemically induced IL-2 signaling complex (CISC) for persistence; and express a chimeric antigen receptor (CAR) specific to a gut antigen for localization, called GNTI-932. Here we evaluated the biological utility of our gut-specific CAR to support EngTreg gut localization and boost antigen-specific suppressive activity for the treatment of IBD. Murine CAR-EngTreg (CAR-mEngTreg) surrogates were generated and top candidates determined by an *in vitro* activation and suppression assay. Target specific CAR-mEngTregs rapidly homed to the gut over non-target tissue sites and were more proliferative, compared to non-target-CAR controls in naive mice. Intravascular staining revealed reduced levels of circulating target specific CAR-mEngTregs in the vasculature of off-target tissues, particularly the lung. Preferential gut-localization and proliferation was also observed in an inflammatory model of acute colitis (Dextran-sodium-sulfate). Consequently, target specific CAR-mEngTreg cells significantly accelerated resolution of pathology in a CD45RBhi colitis model, characterized by reduced accumulation of inflammatory immune cells, biomarkers (lipocalin-2) of gut inflammation and histopathological scores. These data demonstrate our CAR-EngTreg strategy recognizing a gut specific target endows superior localization and efficacy profiles in preclinical models of colitis to enhance Treg therapies for IBD.

W173. Influence of SLAM Family Receptors in the NK Cell-mediated Surveillance of Lymphoblastic Acute Leukemia

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Abstract Text: Acute lymphoblastic leukemia (ALL) represents the most common pediatric cancer worldwide. NK cells along other lymphocytes including innate lymphocytes cells (ILCs) have important functions in recognizing and eliminating malignant cells. As consequence, NK cells are crucial for developing novel therapeutical treatments of various cancers including leukemia. Members of the Signaling Lymphocyte Activation Molecule (SLAM) family are surface molecules expressed in hematopoietic cells where they regulate distinct cell responses. An altered expression and/or function of these receptors may be involved in the etiology of several diseases. Here we describe that the overall expression of members of the SLAM family is altered in leukemia cells form pediatric patients. In addition, the solely expression of a single type of a SLAM receptor in leukemia target cells was sufficient to enhance the release of cytotoxic granules content in primary NK cells. Moreover, coating the leukemia cell surface with specific engagers containing recombinant SLAM family receptors (SFR) was sufficient to enhance NK cell degranulation and unleash the cytotoxic competence of primary NK cell from ALL patients. Finally, NK effector responses promoted by SLAM receptors engagement were dependent on the capability to recruit PLC-□. These results suggest that SFR are an important resource for the treatment of pediatric acute lymphoblastic leukemia.

Immunogenetics

Th151. Germline Variants and Somatic Mosaic Chromosomal Alterations Affect COVID-19 Vaccine Immunogenicity, Infectious Diseases, and Autoimmune Diseases

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Abstract Text: Vaccine immunogenicity is influenced by the vaccinee's genetic background. We performed GWAS of vaccine-induced SARS-CoV-2-specific IgG antibody titers and T cell immune responses in Japanese cohorts (mRNA-1273 and BNT162b2, n=2086). We identify associations with SARS-CoV-2-specific antibody titers at the immunoglobulin heavy chain (IGH) and MHC locus, and associations with the T cell responses at MHC. The lead variants at IGH contain a missense variant (rs1043109-C; p.Leu192Val) in the Immunoglobulin Heavy Constant Gamma 1 gene (IGHG1), which strongly decreases the antibody titers (β=-0.54) and is rarely present in Europeans, highlighting the importance of genetic studies in various populations. SARS-CoV-2-specific antibody serostatus data from the UK Biobank (UKB; N=152,906) independently reveal associations at the IGH, MHC, immunoglobulin kappa light chain and 16p11 locus. HLA imputation analyses in the Japanese cohorts pinpoint HLA-DRB1 as the strongest determinant of the antibody titers, and HLA-DRB1 and HLA-DPB1 as that of the T cell responses within MHC. Blood proteome profiles (2,932 Olink Explore proteins) from 4,236 individuals demonstrate that antibody titer-associated variants modulate circulating immune regulators, including LILRB4 and FCRL6. In addition to germline mutations, age-related somatically expanded mosaic chromosomal alterations (mCA) affecting MHC and IGH impair SARS-CoV-2-specific antibody production. Furthermore, MHC/IGH-affecting expanded mCAs confer risk of infectious and immune diseases, such as sepsis and Graves' disease, in Japanese and European biobanks (Ntotal=663,133). Expanded mosaic loss of sex chromosomes (X or Y) impact such phenotypes including rheumatoid arthritis. Our results underscore the contribution of both germline and somatic mutations to adaptive immunity functions.

Th152. Whole Genome Sequencing in 25 Families with Suspected Inborn Errors of Immunity: Diagnostic Yield and Clinical Relevance of Genome-wide Analysis

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Abstract Text: Introduction Inborn errors of immunity (IEIs) represent a broad spectrum of 485 disorders caused by more than 500 different responsible genes, most of which are known to be monogenic. Next-generation sequencing-based exome sequencing has facilitated the identification of causative genetic variants in IEIs, achieving diagnostic yields of up to 40%. Methods We analyzed data from 25 probands with suspected IEIs based on clinical phenotypes, recruited as part of National Bio-Big Data Program's whole genome sequencing (WGS) project between July 2020 and February 2022 from Samsung medical center. The analysis followed a sequential approach, starting with candidate gene panel analysis to identify both small variants and structural variants, then progressing to a genotype-driven strategy using in-house pipelines. The pathogenicity of the identified variants was evaluated by clinical and laboratory manifestations and presence of family history. Results Causative variants were identified in 10 (40%) probands through candidate gene panel analysis including BTK, CYBB, DKC1, DNAH11, DNAH5, IL2RG, NFKB2, PIK3CD and SH2D1A, in two (8%) proband through genotype-driven analysis including NF1 and PTPN11, and in three (12%) probands through structural variant analysis including BTK and LRBA. Overall, the pathogenic or likely pathogenic variants implicated in monogenic IEI were detected in 60%. Four variants of uncertain significance were identified in three probands (12%). The structural variants were confirmed by RNA sequencing or protein expression.

Conclusion WGS enabled the effective identification of causative genetic variants including complex structural variants. This indicate a potential diagnostic gain by performing WGS from patients with suspected IEIs.

Tu165. Beyond Monogenic Models: Genetic Influences on Immunodeficiency Penetrance in TACI Variant Carriers

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Abstract Text: Common variable immunodeficiency (CVID) is the most prevalent primary immunodeficiency (PID). While PIDs are generally considered monogenic diseases, fewer than 10% of CVID patients receive a monogenic diagnosis suggesting that many cases may not be monogenic in origin. The p.C104R and p.A181E variants in TACI, which regulates B cell number and function, are associated with CVID and found in approximately 7% of patients. However, as these variants are present in about 1% of healthy individuals, they cannot solely cause the disease. Why then, do some carriers of these TACI variants develop CVID, while others remain unaffected? To investigate the role of additional genetic variants on the variable penetrance of immunodeficiency in individuals with TACI p.C104R and p.A181E, we analysed whole-genome sequencing data from 886 individuals with PID, including 51 carriers of TACI variants, and another 160 TACI variant carriers without PID. We assessed the burden of common variants (minor allele frequency [MAF] > 5%) using polygenic risk scores, and rarer variants (MAF < 3%) in digenic combinations using the supervised machine-learning approach Variant Combinations Pathogenicity Predictor. Our analysis revealed that common genetic variation does not drive antibody deficiency in TACI variant carriers. However, we identified several candidate modifying variants within the underexplored 1-3% MAF range. Notably, some of these variants are predicted to be deleterious in genes essential for B cell function, such as TNFRSF13C (the BAFF receptor). This work highlights the role of non-Mendelian mechanisms in the genetic penetrance of TACI p.C104R and p.A181E and identifies potential novel modifiers.

Tu166. Deciphering State-dependent Immune Features at Single-cell Resolution from Multi-layer Human Omics Including Transcriptomics, Germline Variants, Mosaic Chromosomal Alterations, and Plasma Proteomics

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Abstract Text: Multi-omics molecular quantitative trait locus (mQTL) analyses elucidate biological mechanisms of human diseases, while current mQTL catalogues are mostly at bulk resolution and centered on Europeans. Here, we constructed immune single-cell atlas with multi-layer omics from 235 Japanese, including COVID-19 patients and healthy subjects. Dataset consists of single-cell transcriptomics from >1.5 million peripheral blood mononuclear cells, host genetics, plasma proteomics, and gut metagenomics. We mapped the germline genetic effects on gene expression within immune cell types and across cell states. We elucidated cell type and context-specific HLA and genome-wide associations with T/B cell receptor repertoires (e.g., COVID-19 and HLA class I interactions in $CD8^+$ T cells). Colocalization utilizing dynamic genetic regulation of gene expression provided better understandings of genome-wide association signals. Differential gene and protein expression analysis depicted cell type and context-specific effects of polygenic risks. Leveraging whole-genome sequencing and SNP genotyping, we projected various

somatic mutations such as mosaic chromosomal alterations (mCAs), loss of Y chromosome (LOY), and mitochondrial DNA heteroplasmy into single-cell resolution, which were highly cell type-specific. We identified immune features specific to somatically mutated cells (e.g., monocyte-specific accumulation of LOY). *In vitro* analysis of clonally expanded mCAs in a COVID-19 patient using single-cell B cell receptor showed no reactivity against SARS-CoV-2 antigens. Single-cell technology resolved associations between disease-related gut bacteria and peripheral immune cells abundance. Thus, immune cells were dynamically regulated in a cell state-dependent manner characterized with multi-omics profiles. Our study shows a value of multi-omics anchored by single-cells to interpret biological phenomena at fine resolution.

Tu167. Dr. Jeykyll and Mr. Hyde- the Curious Case of the Mixed and Overlap Disease in Rheumatology; Immunological Perspective

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Abstract Text: Immunogenetics plays a vital role in the expression of rheumatologic diseases. The HLA is the critical link between the putative auto-antigen, the subsequent T cell cytotoxicity, and the B cell autoantibody formation. This is reflected in the clustering of patients into “B” and “DR” groups of inflammatory disorders—the spondyloarthritis group with the HLA-B27 allele, the rheumatoid arthritis group with the HLA-DRB-01,04, etc. However, immune-response genes like the PTPN22, ERAP, and other genes associated with cellular and molecular machinery of the immune response can be the more important “final common pathways” associated with immune pathology. Part 1 of the talk will discuss the findings from the cohort at our center and describe the” overlap musculoskeletal disorder”- patients with contrasting forms of inflammation in single patients, or their clustering in first-degree relatives. This relates to the diagnosis of rheumatoid arthritis which is a prototypical autoimmune inflammatory disorder and spondyloarthritis which is an autoinflammatory inflammatory disorder. The possible heritable immune and cellular/molecular mechanisms for this unique scenario will be discussed through illustrative case summaries and treatment effects. The related phenomenon of “unmasking” phenotypic traits when patients are treated with targeted therapies and how this relates to “overlap disorders” will be part 2 of my talk. Illustrative case scenarios from my psoriatic arthritis cohort and the research agenda arising from this will be added to the discussion will be discussed.

W174. Enhancer Hijacking of IgH Locus Leading to Antibody Deficiency and Invasive Streptococcus Pneumoniae Infection

Joshua Tobin[1], Matthew Du[1], Changxu Fan[1], Tony Yao[1], Birk Evavold[1], Alex Paul[1], Ana Kolicheski[1], Jennifer Poursine-Laurent[1], Nermina Saucier[1], Rachel Bielecki[1], Yongjun Wang[1], Kristen McDaniels[1], Tomoki Kawai[2], David LaFon[3], John Kuster[4], Peggy Kendall[1], Caroline Horner[1], Naresha Saligrama[1], Luigi Notarangelo[2], Richard James[5], Stuart Tangye[6], Ting Wang[1], Jeffrey Bednarski[1], Neil Romberg[7], Ali Ellebedy[1] and Megan Cooper[1]

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Abstract Text: We investigated two families with an autosomal dominant pattern of invasive Streptococcus pneumoniae infection associated with specific antibody deficiency (SAD). In Family A, two male siblings presented at < 5 years with S. pneumoniae sepsis. The father had a history of meningitis and recurrent pneumonias, and a female

sibling had milder infections. In Family B, a male presented during infancy with S. pneumoniae meningitis. All 5 individuals had significantly reduced memory B cells, and tested patients had normal serum immunoglobulin levels but poor responses to S. pneumoniae vaccination or infection. Primary B cells from patients had normal BCR spectratyping but diminished *in vitro* plasmablast differentiation. Whole genome sequencing identified similar ~650kb tandem duplications on Chr14q32.2 in all 5 individuals, encompassing part of the IGH locus and upstream genes, with complete penetrance in affected individuals. Bulk and scRNA-seq revealed B-cell-specific overexpression (100-fold) of one duplicated gene, JAG2, encoding the Notch ligand jagged-2. Long-read sequencing and Hi-C demonstrated the IGH locus interacting with JAG2 due to the duplication, supporting enhancer hijacking leading to overexpression of JAG2. Enhancer hijacking is well-described in cancer but has never been described in IEI. Overexpression of human JAG2 led to a defect in marginal zone B cell development in a bone marrow chimera. Together, these findings demonstrate a novel genetic mechanism of IEI, a new role for JAG2 in B cell differentiation, and a genetic cause of SAD. Ongoing work is focused on the effects of JAG2 overexpression on B cell function and an *in vivo* vaccination model.

W175. Functional Assessment of Genetic Variants of CARD11 and BCL10 with Saturation Genome Editing

Nathan Camp[1], Nick Moore[2], Nahum Smith[3], Alan Rubin[2], Lea Starita[3], Richard James[3] and Samantha Dawson[1]

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Abstract Text: The effective use of genomic medicine is often hindered by variants of uncertain significance - genetic variants in disease-associated genes whose impact on disease development remains unknown. The CARD11-BCL10-MALT1 signalosome plays a critical role in B and T cell function, with each gene implicated in various immune-related diseases. Loss-of-function in these genes are linked to immunodeficiency and, in some cases, primary immune regulatory disorders, while gain-of-function is associated with lymphoma. In this study, we used cloning-free saturation genome editing to introduce all possible single nucleotide variants of the coiled-coil domain of CARD11, and the CARD domain of BCL10 into human primary T cells and a B cell line. Using a proliferation based assay we were able to functionally score 3,022 genomic variants spanning 321 amino acids of the coiled-coil domain of CARD11 and 2,178 variants covering 234 amino acids of the coding region of BCL10. We were able to show that loss-of-function variants that clinically present as primary immune regulatory disorders, such as those in CADINS Disease (C223T and R235P) or atopic dermatitis (L194P), were scored as loss-of-function in the T cell assay. Known variants associated with gain-of-function diseases such as lymphoma (L245P, S250P) were not distinguishable in the T cell assay, but were scored as gain-of-function in B cells treated with inhibitors of B cell receptor signaling. Collectively, these data demonstrate how saturation genome editing can be used to score and classify variants of uncertain significance in genes associated with immune disease.

W177. Meta-analysis of Genome-wide Association Studies of Asthma Exacerbation

Michael Chao, Hamid Mattoo, Samuel Lessard, Zhaohui Du, Mahasweta Bhattacharya, Tejaswi Iyyanki, Emanuele de Rinaldis, Shameer Khader and Clement Chatelain

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Abstract Text: Asthma exacerbations are a major cause of asthma-related morbidity and mortality. Pathogenesis of asthma involves a complex interaction between genetic and environmental factors. Genetic factors for susceptibility may differ from those associated with progression and severity, suggesting the presence of genetic factors specific to exacerbations. Genome-wide association studies (GWAS) have identified ~200 loci associated with asthma susceptibility, but few studies have investigated genetics of underlying exacerbations. Here, we present a large-scale GWAS of asthma exacerbations using a meta-analysis framework including FinnGen and UK Biobank. Individuals with asthma and at least one exacerbation were compared to those with asthma but no history of

exacerbation. Exacerbations were defined based on physician or hospital records, prescriptions for oral corticosteroids and antibiotics. UK Biobank included 11,798 exacerbators and 30,543 non-exacerbators, while FinnGen included 8,469 exacerbators and 38,518 non-exacerbators. Individual GWAS was performed using REGENIE adjusted for age, sex, ancestry and relatedness. Meta-analysis was conducted using fixed-effect inverse variance-weighted method implemented in METAL. We identified 23 independent signals, four of which reached genome-wide significance (p< 5x10-8). Potential causal genes for asthma exacerbations were prioritized using a combination of statistical approaches. Inflammatory pathways activated in response to infectious agents are implicated, which can cause acute lung injuries and subsequently contribute to exacerbations in asthma patients. Validation of these findings in independent cohorts and across diverse ancestries, coupled with functional investigations, will be essential to elucidate their mechanisms of action and potentially inform target discovery and therapeutic development.

W179. X-MAID Disease Caused by a Novel Synonymous MSN Variant That Disrupts mRNA Splicing

Simran Samra, Pariya Yousefi, Bhavi Modi, Jessica Halparin, Scott Cameron, Catherine Biggs and Stuart Turvey

BC Children's Hospital, University of British Columbia, Vancouver, BC, Canada

Abstract Text: Introduction: MSN encodes moesin, a cytoskeletal adaptor protein that plays a critical role in maintaining cell rigidity and is primarily expressed in lymphocytes and endothelial cells. Missense and premature stop variants in the MSN gene are known to cause X-linked moesin-associated immunodeficiency (X-MAID), a rare, sex-linked disease. Methods: Clinical assessments and a targeted gene panel were performed. The identified MSN variant was segregated through the family. RNA was isolated from blood samples and then sequenced. RNA-sequencing results were validated using quantitative PCR. Results: We identified two brothers with a hemizygous, synonymous variant in MSN (NM_002444.3: c.795G>A, p.Pro265=) that was predicted by in silico prediction tools to be damaging (CADD score of 25) and likely to alter mRNA splicing (SpliceAI delta score for donor loss was 0.82). The brothers inherited the variant from their healthy, carrier mother. The brothers presented with a very similar phenotype of severe lower leg dermatitis, chronic non-healing ulcers, clinical features of venous insufficiency, hypogammaglobulinemia, and mild lymphopenia. RNA sequencing revealed aberrant splicing of the MSN transcript in the brothers, consisting of either a complete skip of exon 7 or the retention of intronic sequences that result in a premature stop codon. These aberrant splicing events were observed at low levels in the carrier mother and were absent in the healthy controls, and were validated using quantitative PCR. Conclusion: This study is the first to report X-MAID caused by an MSN splicing variant and further emphasizes the possibility that synonymous variants can be disease causing.

Immunology Across the Ages

Th153. Mechanisms of Inflammation Across the Lifespan in Individuals with down Syndrome

Felipe ten Caten[1], Fernanda Coirada[1], Fernanda Bruno[1], Perla del Rio Estrada[1], Jackeline Malheiros[2], Cassia Terrassani Silveira[2], Luciene Covolan[2] and Susan Ribeiro[1]

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Abstract Text: Aging is accompanied by increased systemic inflammation, a decline in immune responses, and the appearance of several comorbidities. Down syndrome (DS), caused by the trisomy of chromosome 21, presents hyperinflammation across the lifespan, resulting in accelerated biological aging. We examined age (15-60 years old) and sex-matched DS and non-DS controls (n=120) to explore immune dysfunction and aging mechanisms. Multi-

omic approaches were applied to cells and plasma samples (bulk RNA-seq, flow cytometry, cytokine array, untargeted metabolomics). Linear and spline functions were fitted to the data to identify age-associated features and differences between groups. We observed that individuals with DS presented increased metabolites related to amino acid catabolism and higher levels of inflammatory cytokines/chemokines (IP-10, IL-10, MIP3 and MCP families) throughout their lifespan. Furthermore, monocytes and natural killer cells from these individuals presented significantly higher expression of post-translational modifications in histones associated with transcriptional activation (H3K9Ac, H3K4me3), indicating a dysregulated profile of trained immunity, which was followed by higher frequencies of inflammatory monocytes (NLRP3, NLRX1, IL1-b, Caspase-1). This hyperactivation was also observed in cells from the adaptive immune compartment, resulting in immune exhaustion (IRF4, TOX). Pathways associated with several diseases (endocarditis, stroke, sepsis) were also significantly upregulated in individuals with DS across ages as compared to non-DS individuals. Understanding the mechanisms at early stages in life related to comorbidities, and common across DS and the aged population, could lead to interventions to modulate those pathways and delay and/or reverse these comorbidities, resulting in better quality of life for the population in general.

Th154. Ontogeny of Plasma Cytokine and Chemokine Concentrations Across the First Four Months of Human Life in a Papua New Guinea Cohort

Thomas Kouyate[1], Athena Nguyen[1], Rebeca Ford[2], Alec Plotkin[1], Geraldine Masiria[2], Joe Jude[2], Joann Arce[1], Oludare Odumade[1], Beate Kampmann[3], Tobias Kollmann[4], Peter Richmond[4], Ofer Levy[1], Anita van den Biggelaar[4], William Pomat[2] and Kinga Smolen[1]

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Abstract Text: Dynamic changes in plasma immune biomarkers in early life follow a robust ontogeny as infants adapt to their new environment. We previously reported that immunomodulatory plasma cytokines and chemokines demonstrate dynamic ontogenetic patterns across the first week of life in neonates from The Gambia. However, whether these changes are generalizable to other regions of the world remains unknown. Here, we examined plasma cytokine and chemokine in Papua New Guinea healthy infants (n=87) across the first four months of life. Using a multiplex assay, 48 cytokines and chemokines concentrations were measured in heparinized peripheral blood plasma samples collected at day of life (DOL) 0, 7, 30, and 128. Unsupervised clustering was used to identify ontogeny patterns in early life. In this cohort, several cytokines and chemokines that shape cellular immunity increased in concentration over the first four months of life, including CXCL10, IFNγ, and IL-2. Contrastingly, other cytokines and chemokines showed reductions, including CXCL8, IL-6, and TGFα. These findings did not appear to be affected by birth season, gestational age, sex, or maternal age. These results validate previously observed patterns in The Gambia, suggesting a universal baseline of cytokine and chemokine trajectories in early life, and illustrating a key role of human immune ontogeny. Understanding early life trajectories of plasma cytokines and highlighting conserved patterns among different geographic populations likely indicates important pathways for human immune development. This may inform novel approaches to diagnosis, treatment, and disease prognosis. Future studies should investigate which cytokines and chemokines deviate in different disease states.

Th187. Hypo-functionality of Fetal Tregs Allow for a Permissive Regulatory Environment for Gestational T Effector Memory Maturation in Humans

Joo Guan Yeo[1], Naomi McGovern[2], Archita Mishra[3], Martin Wasser[1], Pavanish Kumar[4], Shi Huan Tay[4], Sharifah Nur Hazirah[4], Xiu Qi Tan[4], Nursyuhadah Sutamam[4], Farah Nadiah Azman[4], Camillus Jian Hui Chua[4], Phyllis ZiXuan Chen[4], Fauziah Ally[4], Charles-Antoine Dutertre[5], Lakshmi Ramakrishna[4], Su Li Poh[1], Liang Xie[4], Yiping Fan[6], Catherine Donner[7], Maria Papadopoulou[7], David Vermijlen[7], Thaschawee Arkachaisri[6], Jerry Kok Yen Chan[6], Florent Ginhoux[8], Salvatore Albani[1] and Jing Yao Leong[1]

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Abstract Text: The gestational environment is frequently perceived to be predominantly suppressive and incompatible for T effector function maturation. However, evidence of a competent effector fetal environment in humans has been mounting. Herein in this study, we employed a high parametric, mass-cytometry based approach to investigate the fetal circulatory and micro-environmental immunomes, with an aim to understand the inception and extent of fetal effector T cell priming and its relation with regulatory mechanisms. We uncovered evidence of fetal thymic immune imprinting, coupled with both systemic and tissue effector memory development in human fetuses. Correspondingly, in the regulatory compartment, we detected the unique presence of thymic Tbet^{+}Treg in fetal tissues at elevated levels as compared to adult tissues. Intriguingly, fetal Tregs in lung tissues, though capable of suppression, were hypo-suppressive as compared with adult counterparts. We discovered that a proportion of fetal Tregs lost FoxP3 commitment during expansion and exhibited higher TCR clonatype sharing with effector T cells, altogether reflecting higher plasticity in fetal Tregs than adult. Furthermore, epigenetic profiling of the FoxP3 promoter locus reveals that fetal Tregs were only partially de-methylated resulting in lineage instability. In summary, our data provide evidence of a gestational regulatory environment that is permissive for T effector functional maturation due to relative instability and hypo-functionality of fetal Tregs.

Tu168. Age-Related Patterns of Type II Interferon Immunity: Implications for Intramacrophagic Infections and MSMD Diagnosis During Childhood

Yiyi Luo[1], Aina Freixedas[1], Guillermo Argüello[2], Daniel Acevedo[1], Cristina Jou[1], Anna Codina[1], Jesús Márquez[1], Alexandru Vlagea[3], Sara Peiró[3], Víctor Bolaños[3], Ángela Deyà[1], Ana García-García[1], Celia Martí[1], Manel Juan[3], Ana Esteve-Solé[4] and Laia Alsina[1]

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Abstract Text: Type II interferon (IFN) immunity is crucial for controlling intramacrophagic infections, driven by the interaction between innate immunity (macrophage-derived IL-12) and adaptive immunity (Th-derived IFN-γ). This study examines the maturation of type II IFN immunity in 55 healthy children (ages 1–18) to enable proper identification of deficiencies as part of the diagnostic evaluation of Mendelian Susceptibility to Mycobacterial Diseases (MSMD). The IL-12/IFN-γ axis was assessed through: 1) cytokine production after mycobacterial stimulation (Luminex and ELISA for IFN-γ, IL-12p70, TNF, IP-10, IL-1RA, IL-10, IL-1β and IL-6), 2) IFN-γR1/R2 expression on monocytes, and 3) STAT1 phosphorylation/dephosphorylation. T cell maturation, as the primary IFN-γ source, was evaluated via extended immunophenotyping (naive/memory/activated, Th1; Th2; Th17; Th1/17; Tfh) and proliferation assays. Main findings: 1) stable expression/production of key components of the IL-12/IFN-γ axis (IFN-γ, IL-12, TNF, IFN-γR1/2, and STAT1 activity) across ages confirming the stability of innate immune function throughout childhood; 2) increasing responses to IFN-γ with age reflected by increased IP-10 production, and

increased in the IFN-γ counter-acting anti-inflammatory cytokines (IL-10, IL-1RA); and 3) progressive T cell maturation, including Th1, Th17 and Th1/17 subsets, with significant milestones between 6–8.6 years, while T cell proliferative capacity remained stable. These observations highlight the stability of IL-12/IFN-γ axis innate components with age, accompanied by enhanced downstream IFN-γ signaling, aligning with the maturation of Th cell compartment. These underscored the limited need for age-specific evaluation of IL-12/IFN-γ axis innate components in MSMD diagnosis, while emphasizing the importance of T cell profiling due to its progressive maturation.

Tu169. Characterizing a Novel IRF8 Variant Through a Multidisciplinary Approach

Crescent Isham[1], Hyoungjun Ham[1], Elizabeth Ristagno[1], Cristina Correia[1], Scott Ennis[1], Richard Kandasamy[1], Kishore Garapati[1], Cheng Zhang[1], Mindy Kohlhagen[1], Elham Sadighi Akha[1], Maria Rodriguez-Quevedo[1], Destiny Schultz[1], Baoyu Chen[4], Thomas Boyce[1], Seth Gregory[1], Mira Kohorst[1], Surendra Dasari[1], David Murray[1], Kevin Halling[1], Benjamin Kipp[1], Attila Kumanovics[1], Hu Li[1], Akhilesh Pandey[1], Daniel Billadeau[1] and Amir Sadighi Akha[1]

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Abstract Text: The few reported patients with pathogenic interferon regulatory factor 8 (IRF8) variants have manifested two distinct phenotypes: an autosomal recessive severe immunodeficiency with significant neutrophilia and absence/significant decrease in monocytes and dendritic cells; and a dominant-negative form with only a decrease in cDC2 dendritic cells and susceptibility to mycobacterial disease. Genetic testing of a child with persistent EBV viremia identified a novel IRF8 variant: c.1279dupT (p.*427Leuext*42). The variant was also found in his mother, who was subsequently diagnosed with an HPV^{+} tumor. We sought to examine the pathogenicity of the identified IRF8 variant and its phenotypic and functional characteristics. The 42 amino acid C-terminal extension of the mutant IRF8 protein (~4 kDa heavier than wild-type) impaired IRF8 nuclear localization in a dominant-negative manner, and inhibited IRF1/IRF8-mediated transcriptional activities. Both patients had a decrease in plasmacytoid dendritic cells (pDC) and in cDC1 cells, a mild neutrophilia and a mild monocytosis. Their existing pDCs had impaired IFN-□ production. Upon TLR engagement, the production of IL-1□, IL-6, IL-10 and IL-12 by their monocytes, and of IL-12 by their myeloid DCs were within normal limits. NK cell development and cytolytic activity were essentially normal. RNA-seq and proteomic approaches bolstered the phenotypic and functional findings. This study defines the pathogenic nature of the c.1279dupT (p.*427Leuext*42) IRF8 variant, determines its dominant-negative mechanism of action and broadens the existing phenotype of human IRF8 immunodeficiency.

Tu170. Dysregulated Lymphocytes in Infertile Men: Potential Biomarkers for Non-communicable Disease Development

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Abstract Text: Infertile men are at high risk of developing age-related non-communicable diseases (NCDs). Infertile men diagnosed with OligoAsthenoTeratozoospermia (OAT) and Non-Obstructive Azoospermia (NOA) are characterized by a pro-inflammatory milieu with increased frequency of myeloid cells and reduced T cells, which are immune alterations typically observed in healthy elderly men. Distinct T cell signatures of exhaustion and senescence discriminate the two infertile conditions. With the goal of identifying biomarkers to monitor and potentially predict the development of NCDs in infertile men, we further characterized the lymphoid compartments in a larger cohort of patients. While the proportion of lymphoid cells in infertile is reduced compared to that of fertile men, T cells, specifically in OAT patients, are characterized by the expression of the exhaustion markers TIGIT/LAG-3/Eomes/PD-1 alone or in combination. Moreover, a high proportion of T cells in NOA, but not in OAT patients,

expressed high levels of the fluorescent senescent-associated-β-Galactosidase marker. A reduced total and naive B cell frequency, associated with an increased proportion of antibody-secreting cells, both plasma blasts and plasma cells, and of autoreactive cells, is also observed in infertile men, suggesting the hyperactivation of B cells in infertile patients. These findings provide evidence that subtypes of male infertility are characterized by specific immune signatures. More broad correlation analysis of the immunological status, in association with clinical parameters and genomic and transcriptomic data, will define specific signatures to better identify patients at higher risk of earlier developing NCDs and will be instrumental in developing personalized secondary preventive strategies.

W180. Immune Development in Early Life (IDEAL): A Systems Immunology Approach to Identify Endotypes Predictive of Vaccine Responsiveness, Respiratory Infections, and Asthma

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Abstract Text: Background: The IDEaL-Rome prospective pediatric cohort, based at Bambino Gesù Children's Hospital (OPBG, Rome, Italy), is part of a longitudinal observational study supported by NIH/NIAID. The study aims to identify molecular endotypes associated with increased susceptibility to respiratory infection, asthma (AS) and vaccine responsiveness (VR) in childhood using a systems immunology approach. Methods: The study includes six scheduled visits (V1 at 2 months, V2 at 5 months, V3 at 9 months, V4 at 1 year, V5 at 3 years, and V6 at 5 years of age). Clinical data are integrated with multi-omic datasets, including cytokine/chemokine profiles, proteomic, metabolomic, microbiome, and epigenetics, to define developmental trajectories associated with VR, AS, and infection proneness (IP). VR is assessed by specific antibody titers, AS diagnosis is based on the Asthma Predictive Index, and IP is defined as ≥3 respiratory infections and/or severe infections during the first year of life. Results: From April 2023 to November 2024, 273 participants (129 males, 144 females) were enrolled. To date, 210 participants have completed V4. Among these, 37.1% experienced ≥3 infections in the first year. Reported infections included common cold (168 cases), SARS-CoV-2 (51 cases: 37 confirmed, 14 suspected), bronchiolitis (39 cases), bronchitis (45 cases), pneumonia (2 cases), pharyngitis/tonsillitis (53 cases), otitis media (37 cases), and septic osteoarthritis (1 case). Conclusions: The IDEaL-Rome prospective cohort will provide a robust repository of biospecimens as well as clinical, immunologic, and systems biology data enabling multi-omic analyses to define molecular endotypes predictive of vaccine responsiveness, infection susceptibility, and asthma.

W181. Mapping Neonatal Plasma Proteome Changes over the First 2 Days of Life at an Hourly Resolution

Kinga Smolen[1], Tue Bennike[2], Alec Plotkin[1], Jensen Pak[1], Arthur Viode[1], Christoph Schlaffner[1], Olubukola Idoko[3], Oludare Odumade[1], Dylan Tabang[1], Rym Ben-Othman[4], Joann Arce[1], Al Ozonoff[1], Judith Steen[1], Tobias Kollmann[5], Beate Kampmann[6], Ofer Levy[1] and Hanno Steen[1]

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Abstract Text: Birth represents a dramatic shift from a quasi-sterile intrauterine life to an antigen-rich extrauterine environment. The transition from fetal immune tolerance to facing new immune challenges necessitates a significant immune system adaptation. Key components of the immune system circulate in plasma such that early adaptations are reflected by changes to the plasma proteome composition. We utilized a high-resolution liquid-chromatography and mass spectrometry (LC-MS) platform to define the proteomic changes that occur hourly during the first 48hours of human life. We collected >900 peripheral blood samples from 679 healthy term newborns in The Gambia, West Africa at day of life (DOL)0 and DOL1, calculated the precise time duration between blood collection and birth enabling mapping of the postnatal plasma proteome at hourly resolution. Using LC-MS-based plasma proteomics and multiplex cytokine assays, we characterized 324 proteins and cytokines unveiling distinct and dynamic changes in developmental proteome trajectories. Notable changes included fluctuations in the levels of heme proteins, fibrinogens, serpins, and orosomucoid. Many changes could not be accurately captured at the DOL resolution, suggesting the importance of the hour-of-life in studies focused on the first days of life. Additionally, we identified critical ontogenetic periods after birth in healthy newborns, independent of the 24-hour period, which provide support for an immune clock extending beyond pregnancy. Our study of the neonatal plasma proteome at hourly resolution provides insights into protein ordering, co-regulation, and critical periods immediately after birth highlighting the importance of time in future studies investigating immunity and antigen response in early life.

W182. Non-linear Trajectories of Human Immune Ageing Associate with Long Term Survival

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Abstract Text: Ageing is associated with increasing immune dysfunction, characterised by limited protective responses and persistent low grade inflammation. Our understanding of changes that occur and which may be adaptive or harmful remains limited. Here we quantify multiple immune traits in 10 data modalities across 6,748 individuals aged 18-107 using a total of 24,509 cross-sectional and longitudinal samples. We find highly stereotyped, non-linear changes with a consistent inflection point around 70 years of age. We applied a machine learning model to the integrated dataset to learn and independently validate 13 latent normative trajectories in the data, 12 of which vary with age. We show for two trajectories that an individual's 'immune age', defined as their individual deviation from the population trajectory, is associated with long term mortality independent of age, sex or comorbidity with hazard ratios greater than those seen for cancer or sex and similar to that of cardiovascular disease. Interpretation of features defining immune age trajectories indicated that precipitous contraction of the naive B cell compartment, increased IgM^+ memory differentiation and IgA^+ class switch recombination were strongly associated with shorter survival. In contrast, development of poly-autoreactivity in the absence of clinical autoimmunity appears adaptive,

associating with higher survival rates. Our findings systematically characterise non-linear trajectories of human immune ageing and give fundamental insights into the interacting mechanisms associated.

Immunometabolism

Th155. Dissecting the Glycolytic Requirements of Human T Cells via G6PC3 Deficiency

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Abstract Text: The glycolytic requirements of T cells *in vivo* in humans remain elusive, in part, due to the impossibility of prolonged inhibition of glycolysis. We tackle this limitation by studying glucose-6-phosphatase catalytic subunit 3 (G6PC3) deficiency, a monogenic immunometabolic disorder that causes intracellular accumulation of the metabolite 1,5-anhydroglucitol-6-phosphate (1,5-AG6P) in immune cells. We demonstrate that 1,5-AG6P accumulation markedly impairs glycolytic activities in G6PC3 CRISPR/Cas9 knockout Jurkat T cells and in primary T cells from G6PC3-deficient patients. These findings and our unique cohort of patients with G6PC3 deficiency allowed us to examine how impaired glycolysis impact human T cell responses. Multidimensional analysis using CyTOF shows that G6PC3-deficient patients display lower frequencies of naive T cells with a concomitant increase in the effector memory T cell and TEMRA proportions in both $CD4^+$ and $CD8^+$ compartments. Patients also have an increased frequency of TH2/TH17 ($CD4^+$CD45RA-CXCR3-) cells and indications of impaired thymic function, showcasing the importance of glycolysis for T cell development and differentiation. Furthermore, we show the correlation between T cell glycolytic activities and PD-1 expression, since patient memory T cells exhibit elevated PD-1 expression, and CD3/CD28 stimulation induces a significantly greater upregulation of PD-1 expression on $CD4^+$ and $CD8^+$ T cells from patients than those from healthy donors. An integrative analysis of scRNA-seq and scTCR-seq data shed light on potential mechanisms underlying these observations. Collectively, this study highlights the importance of studying rare genetic diseases as an approach to interrogate the links between cellular metabolism and human immune cell fate and function.

Th156. Genetic Variation of the Alzheimer's Disease Risk Gene CD33 Modifies Microglial Immunometabolism

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Abstract Text: The innate immune system has emerged as a central component of Alzheimer's disease (AD), with more than 75% of AD genetic loci being key innate immune genes. One such AD risk gene is CD33: a transmembrane, sialic-acid binding protein that is specifically expressed by microglia in the brain. Previous studies suggest that CD33 regulates the inflammatory response by modifying microglial metabolism and oxidative phosphorylation. However, it remains unknown how CD33 genetic variation regulates microglial metabolism and how this contributes to AD pathogenesis. To address this question, we generated human microglia-like cells from primary monocytes with different CD33 genetic backgrounds and measured their respiratory bioenergetics. Notably, we

found that the balance of ATP production via oxidative phosphorylation and glycolysis varies significantly with CD33 genotype. Compared to the AD-risk genotype, those with the AD-protective genotype had higher glycolytic activity but lower glucose uptake, specifically due to higher hexokinase activity, while no difference in lactate production or secretion was observed. To gain mechanistic insight, we used a mass spectrometry-based approach to identify binding partners of the CD33 sialic acid binding domain, revealing a novel interaction between CD33 and the glucose transporter GLUT1. This result was further validated by PLA and co-IP in a human monocytic cell line. Finally, using brain bulk RNA sequencing data from the ROSMAP cohort of AD patients and healthy elderly controls, we found CD33:GLUT1 gene interaction to be significantly associated with disease, underscoring the importance of this novel link between genetic, immune, and metabolic risk factors in AD.

Th157. Kynurenine-driven Metabolic Adaptations Rewire CD4 T Cells in a Subset-specific Manner in Human Critical Illness and Severe Sepsis

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Abstract Text: Host immunity in sepsis is characterized by the progressive development of immunosuppression, particularly among CD4 T cells, leading to serious secondary infections, impaired organ recovery, and significant mortality. Bioenergetic dysfunction is archetypal in sepsis, but its mechanistic link to human CD4 T cell dysfunction remains unclear. To investigate, we generated a 644,147-cell scRNA-seq map of critical illness from PBMCs collected from ICU patients at Vanderbilt. In ICU patients, particularly with sepsis, regulatory T cells (Tregs) were preserved in frequency and function while conventional CD4 subsets (Tconv) underwent substantial attrition, reflecting progressive immunosuppression. Reaction-level metabolic modeling by in silico flux balance analysis identified elevated kynurenine metabolic signatures in septic Tregs compared to Tconv. Kynurenine is a biomarker of disease severity in sepsis, but the mechanisms underlying this are unclear. Using an *ex vivo* single cell energetic assay (SCENITH), we demonstrated that ICU patient Tregs exhibited higher basal metabolic rates, increased metabolic plasticity with bolstered glycolytic capacity, and elevated kynurenine uptake. Acquired glycolytic capacity directly correlated with Treg survival and supported suppressive phenotypes, marked by stabilized TIGIT and FOXP3 expression amid mitochondrial stress. Moreover, Treg glycolytic capacity in critical illness associated with key clinical metrics including higher SOFA scores, worse shock, longer hospitalizations, and mortality. Supplementation of healthy Tregs with kynurenine increased their glycolytic capacity, whereas pharmacologic kynurenine 3-monooxygenase inhibition partially reversed the suppressive adaptations of septic Tregs. Collectively, these findings establish immunometabolic dysfunction as a driver of CD4 T cell remodeling in sepsis and highlight therapeutic opportunities to restore immune balance.

Th158. The AhR Agonist Tapinarof Suppresses Human Skin Inflammation by Inducing Metabolic Reprogramming and Reducing Production of Mitochondrial Pro-inflammatory DAMPs

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Abstract Text: The topical AhR agonist tapinarof effectively treats both psoriasis and atopic dermatitis but there are no mechanistic studies of its effects in human skin. We treated inflamed human skin grafts carried by NSG mice for 3 weeks with topical tapinarof versus vehicle control. Tapinarof reduced T cell, macrophage, DC and NK cell activation. Upstream DAMP sensing via cytoplasmic nucleic acid sensing was reduced, leading to reduced cGAS/STING, and inflammasome activation, decreased type I, II, and III IFN and type 1, type 2 and type 17 cytokine

production. *In vitro*, in human T cells, tapinarof reduced mitochondrial ROS production and release of mtDNA into the cytoplasm, impairing type I IFN signaling and reducing T cell activation and proliferation. Treatment of psoriatic skin constructs led to metabolic reprogramming, including inhibition of hypoxia sensing, glycolysis, fatty-acid (FA) b-oxidation, and glutamate influx. Tapinarof-induced AhR signaling reduced HIF1α and mTOR expression in T cells, limiting glucose uptake, glycolytic capacity, and FA metabolism, while also impairing mitochondrial respiration and ATP production, producing marked metabolic dysfunction. Finally, tapinarof disrupted mitochondrial function, by reduced mitochondrial membrane potential and mitochondrial mass, suggesting a decrease in mitochondrial fitness. In summary, tapinarof acts at the level of the mitochondria to reduce DAMP sensing via reduced ROS production and mtDNA release, thus markedly reducing inflammatory tone, and by blocking multiple metabolic pathways and impairing mitochondrial function, thus blocking the cells' ability to use and generate energy in the hypoxic skin microenvironment.

Tu171. Colchicine Attenuates Fine Particulate Matter-induced Foam Cell Formation and Human Monocyte Activation

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Abstract Text: Introduction: Exposure to air pollutant fine particulate matter (PM2.5) is an independent risk factor for cardiovascular diseases. PM2.5 can target monocytes and macrophages, disturb their mitochondrial metabolism, and trigger foam cell formation. PM2.5 also potentiates NLRP3 inflammasome activation and IL-1β release, implicated in atherosclerosis. Colchicine is the only FDA-approved anti-inflammatory drug for atherosclerosis. This agent attenuates granulocyte functions, phagocytosis, and NLRP3 inflammasome assembly, potentially modulating atherosclerotic lesions. This study tested the hypothesis that colchicine attenuates PM2.5-induced foam cell formation. Methods: Primary human monocytes isolated from blood were seeded in RPMI medium, supplemented with 10%FBS, and exposed to PBS (Control) or NIST2786-PM2.5 at 100 μg/mL concentration, for 24 h with or without colchicine (5 ng/mL). Data are mean ± SD, and compared through One-way ANOVA, followed by Tukey. Results: Colchicine attenuated PM2.5-stimulated reactive oxygen species generation (Ctrl:1.00±0.07; PM100:1.19±0.14*; PM100+Colc:1.16±0.11, *P=0.017 vs Ctrl) and phagocytosis (Ctrl:1.00±0.12; PM100:1.71±0.30*; PM100+Colc:1.47±0.25*/#, *P< 0.0001 vs Ctrl and # vs PM100). It also reduced lipid accumulation induced by PM2.5 (Ctrl:1.00±0.11; PM100:1.14±0.12*; PM100+Colc:1.00±0.12#, *P< 0.0001 vs Ctrl and # vs PM100), but these effects appeared independent of IL-1β, IL-6, TNF- and m-CSF, mediators whose production the pollutant enhances while IL-10 (Ctrl:1.30±0.31; PM100:731.8±40.70*; PM100+Colc:425.5±176.3*/#, *P< 0.0001 vs Ctrl and #vs PM100) and MCP-1 (Ctrl:118.7±9.27; PM100:2088.0±967.9*; PM100+Colc:1017.0±798.3, *P< 0.0001 vs Ctrl) levels fell with colchicine. Conclusion: Colchicine attenuates foam cell formation and aspects of monocyte activation induced by acute exposure to PM2.5, likely in a manner independent of the NLRP3 inflammasome pathway.

Tu172. Dual Therapy with GLP-1R Agonists and T Cell Modulation Outperforms GLP-1 Alone in Obesity Treatment

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Abstract Text: The global obesity epidemic has intensified metabolic disorders like insulin resistance (IR), type 2 diabetes (T2D), and metabolic dysfunction-associated fatty liver disease (MAFLD), which can progress to steatohepatitis (MASH) and cirrhosis. Chronic systemic inflammation is central to these pathologies. While GLP-1 receptor agonists (GLP1-RAs), such as Semaglutide, offer significant weight loss and glycemic control, their effects are reversible upon discontinuation and do not directly target inflammation or fibrosis in adipose tissue and the liver. Our study introduces nasal anti-CD3 as a novel immunomodulatory strategy to complement GLP1-RAs. In diet-induced obese (DIO) mice, nasal anti-CD3 reduces systemic inflammation, improves glucose metabolism, and restores tissue homeostasis without affecting weight or energy expenditure. Nasal delivery of anti-CD3 antibody localizes in cervical lymph nodes, reprograms T cell metabolism, and expands regulatory T cells (Tregs), reducing pro-inflammatory immune cells in adipose tissue and the liver. In MASH, nasal anti-CD3 reduces liver inflammation, reverses fibrosis, and enhances metabolic homeostasis. Combined with Semaglutide, it amplifies GLP1-RA benefits, improving tissue remodeling, lipid metabolism, and systemic glucose control. These findings position nasal anti-CD3 as a potent adjunct to GLP1-RAs, addressing inflammation and fibrosis while enhancing metabolic outcomes.

W100. Spatial Profiling of the Tumor Microenvironment Using Imaging Mass Cytometry: Unraveling Metabolic Reprogramming and Cell Signaling Dynamics in Cancer

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Abstract Text: The tumor microenvironment (TME) is a complex cellular ecosystem, which in turn influences tumor development and treatment response. Understanding cellular interactions within the TME is essential for elucidating disease progression and advancing immunotherapy. Imaging Mass Cytometry™ (IMC™) is a spatial biology imaging technique that enables deep characterization of the TME. This approach offers scalable and high-throughput acquisition while generating high-quality data without fluorescence-based limitations such as spectral overlap and autofluorescence. We used IMC to map key pathways in metabolic reprogramming and signaling within the TME by utilizing a ready-to-use immuno-oncology IMC panel. This allowed us to investigate processes that regulate energy production, cellular homeostasis and mitogenic signaling pathways. We acquired data using Preview Mode to assess the whole tissue, followed by higher-resolution imaging of selected regions of interest using Cell Mode, or whole tissue section imaging using Tissue Mode. Data analysis revealed the metabolic profile and organization of cells across cancer tissues. Elevated glycolysis and mTOR pathway activation suggested adaptations to hypoxia and anabolic growth in tumors, while interactions between fibroblasts and immune cells highlighted crosstalk within the TME. Unsupervised pixel and hierarchical clustering using MCD™ SmartViewer offered measurement of metabolic activity and signaling pathway activation within tumors. Spatial biology profiling using IMC highlights the interconnected roles these pathways play in promoting tumor survival and resistance to therapies. These findings are crucial for developing future prognostic assessments and have the potential to guide more effective, personalized cancer therapies. For Research Use Only. Not for use in diagnostic procedures.

W183. Multimodal Analysis of IBD Patients Coupled with Machine Learning Identifies Immunometabolism Targets for Potential Therapies

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Abstract Text: Inflammatory bowel disease (IBD) is a chronic immune disease with increasing global burden. Whilst different therapeutic options are available, their efficacy is often limited to only a fraction of the patients, making the identification of novel therapeutic strategies a compelling need. The etiology of IBD entails genetic and environmental factors, involving different cellular processes and pathways. Immunometabolism and the underlying adaptation of immune and stromal cells to their microenvironment, has recently emerged as potential drivers in the onset and progression of the disease. Here, we systematically analyzed over 3000 metabolic genes and observed broad immunometabolism perturbations across bulk and single-cell transcriptomes of IBD patients. Then, we aimed to uncover new strategies to inhibit metabolic reprogramming in IBD. On this ground, we developed MetID, a novel data and analytical framework that leverages the largest integrated multi-modal data resource available in IBD, along with AI/ML methods, to identify promising immunometabolic targets with potential causative role in the etiology of ulcerative colitis (UC) and Crohn's disease (CD). By using MetID, we could surface the pathological role of lipid metabolism in fibroblast and macrophage cells in driving the disease. From our results we identified SPHK1, a sphingosine kinase which belongs to the clinically validated sphingolipid pathway, as novel therapeutic target for UC and CD and differentiated it from S1PR agonists, in silico as well as experimentally. This work highlights the added value of multi-modal analysis to dissect this complex disease and paves the way to the discovery of novel therapeutic opportunities in IBD.

Immuno-oncology

Th159. Cancer-associated ALDH1 Metabolism Enforces Immune Evasion

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Abstract Text: T cell inflamed, “hot” tumors are known to respond better to immunotherapy compared to T cell non-inflamed “cold” tumors. However, defining cancer cell-intrinsic features that portend “hot” versus “cold” tumor microenvironments has proven challenging. Here we developed 2 novel mouse cancer models, generated from the same tumor, which display contrasting “hot” (BBNF2) and “cold” (BBNF9) tumor phenotypes. Co-implantation of tumors resulted in dominant immune suppression levied by immune “cold” tumors. Comparative analysis of these tumors identified that cancer cell-intrinsic expression of ALDH1 was selectively associated with immune “cold” tumors. BBNF9 tumors had low T cell infiltration and diminished dendritic cell (DC) responses. Treatment of DCs with retinoic acid (RA) the main metabolite from ALDH1 enzymatic activity, could suppress DC responses. DC-specific deletion of Retinoid X receptor alpha (RXRa), a key receptor mediating sense of RA, rendered DCs insensitive to RA-mediated suppression, and enhanced DC IL-12 production. Cancer cell-specific deletion of ALDH1 resulted in reduced tumor neutrophil infiltration, enhanced DC response, and slower tumor growth. Collectively, we find that cancer-cell intrinsic ALDH1 confers immune evasive properties to cancers by engaging a neutrophil axis and suppressing antigen presentation.

Th160. cdc1 and Tissue-resident Exhausted CD8 T Cells Drives Disease Control and Checkpoint Blockade Immunotherapy Response in Melanoma

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Abstract Text: Melanoma is the deadliest form of skin cancer, with a rising incidence in Latin American countries. While immunotherapy has significantly improved melanoma treatment and patient survival, a subset of patients remains unresponsive, emphasizing the need to identify factors critical for successful treatment and to understand disease progression. In this study, we evaluated the presence of conventional dendritic cells type 1 (cDC1) and tissue-resident memory (TRM) CD8 T lymphocytes two key cell types in antitumoral responses in the stroma of melanoma patients using multiplex immunofluorescence and artificial intelligence-based image analysis. Remarkably, we identified two TRM CD8 T cell populations: one expressing the progenitor marker TCF1 (TCF1$^+$) and another lacking it (TCF1-). TCF1$^+$ TRM cells exhibited heightened expression of IFN-γ and Ki67, while TCF1- TRM cells showed increased expression of cytotoxic molecules and were near apoptotic tumor cells. Spatial analysis revealed a correlation between cDC1s and TRMs, with TCF1$^+$ TRM/cDC1 pairs enriched in the stroma and TCF1- TRM/cDC1 pairs predominantly located in tumor areas. Notably, these immune populations were organized into structured immune niches near the tumor area in disease-free patients, compared to metastatic patients who don't show these niches. Importantly, in an independent cohort, we observed that both TRM CD8 T cell populations and cDC1 were highly abundant in patients responding to CBI, even before treatment, suggesting their potential use as predictive biomarkers for treatment selection.

Th161. Implementation of Complex *in vitro* 3D Lung Cancer Model to Monitor Resistance to Immune Checkpoint Inhibitors Through Deep Profiling at Single-cell and Spatial Resolution

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Abstract Text: Lung cancer is the leading cause of cancer related deaths worldwide with non-small cell lung cancer (NSCLC) being the most common type (80-85%). Immune Checkpoint Inhibitors (ICIs) has revolutionized the treatment of NSCLC. Nevertheless, some patients show resistance to treatment and knowledge on resistance mechanisms remains limited. The tumor microenvironment (TME) plays a pivotal role in response to immunotherapies. Yet, access to tumors after the first lines of treatment is challenging. To overcome this limitation, we propose to characterize TME influence on ICI resistance developing a complex human NSCLC derived 3D co-culture model including tumor cells, endothelial cells, fibroblasts, T cells and monocytes. We aim to profile the 3D model at baseline and monitor the influence of TME on response to standard treatment (chemotherapy + ICI treatment) through an exhaustive molecular spatial and phenotypic evaluation of the whole immune context. Preliminary data from flow cytometry, confocal imaging and spatial transcriptomics confirmed the presence of all different cell types at D5, with a prevalence for tumor cells. Moreover, at D14 we observed treatment effect in tumor cell killing with low residual immune cells. We are now collecting longitudinal data from D5 to D14 using single cell transcriptomics to better monitor interactions between treatment and TME. Those data are also used to optimize culture conditions to obtain an even more relevant TME model, including hot and cold like TME, to enable the identification of novel targets and biomarkers to overcome ICI resistance and improve patient stratification.

Th162. Regulation and Dysregulation of Disease Specific Immune Responses by Human Dendritic Cell Subset

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Abstract Text: Dendritic cells (DCs) are key antigen-presenting cells that control both immunity and tolerance. Understanding the principles by which DC controls these responses has provided a rich basis for studying and improving clinical outcomes in treating human diseases. Part of the complexity is due to the existence of distinct DC subsets, each bearing different microbial receptors, surface molecules and cytokine expression. The biological raison d'être for separate DC subsets has been the focus of many studies, including ours. Our research focuses on skin-migrated DC2 lineage cells, specifically on two subtypes differentiated by CD5 expression. The $CD5^+$ DCs are highly efficient at priming cytotoxic $CD8^+$ T cells and induce inflammatory T helper cell responses They are elevated levels in inflamed psoriatic skin plaques, where high effector T cell responses are seen, and are reduced in malignant tissues and correlate with cancer patient survival. Consistent with this notion, we demonstrate, using human patient cells and mouse models, that CD5 functions to trigger an inflammatory pathway of DC maturation and T cell activation. Deletion of CD5 on DCs reduces tumor rejection and Immune checkpoint blockade (ICB) immunotherapy response. During successful ICB immunotherapy, $CD5^+$ DCs increased, supporting their interaction with CD5hi T cells. New data on the differentiation of this unique DC type will be presented. Overall, our studies highlight the role of CD5-expressing DCs in immune regulation. They provide insight into how immunotherapies like ICB work and identify CD5 on DCs as a new potential target for enhancing response rates among patients undergoing treatments.

Th163. Restoring Natural Killer Cells with FLT4-targeting Peptide in Acute Myeloid Leukemia

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Abstract Text: The Fms-related tyrosine kinase-4 (FLT4) is involved in AML progression. Previously, we found that dysfunctional natural killer (NK) cells with low interferon-gamma (IFN-γ) in AML are restored by FLT4 antagonist, MAZ51. However, peptides targeting FLT4 in restoring NK cells in AML are not elucidated. We developed peptides targeting FLT4 for clinical application and ultimately selected several peptides to examine whether the frequency of lymphocytes, especially T cells and NK cells, and high expression of IFN-γ were restored by peptide treatments, as shown by MAZ51 treatment in AML syngeneic mouse model. Through *in vivo* experiments, MAZ51, peptide, and cytosine arabinoside (ara-C) were treated. Flow cytometry, elispot assay, and real-time qPCR analysis were performed. Similar with MAZ51, high IFN-γ was expressed in AML-NK cells when using the specific peptide. In addition, T and NK cells can be restored with high IFN-γ in leukemic environment, when peptide was co-cultured with ara-C. Interestingly, the frequency of regulatory T cells significantly decreased by peptide, implying the role of peptide in modulating the AML-related immune niche. When peptide was combined with ara-C, the spleen and liver taken from AML mice showed more effective eradication of AML blasts than single therapy. Based on the results of NK cells' functional recovery by FLT4 inhibitory peptide in AML mice, peptide targeting FLT4 in combination with chemotherapy may inhibit AML progression by blocking the lymphatic markers on AML NK cells. These findings possibly provide some clues for developing a novel immunotherapeutic strategy for standard therapy-resistant patients with AML in clinic.

Th164. Single-cell RNA Sequencing Reveals Conserved T Cell Populations Detected Across Inflamed Kidney Tissue and Urine of Patients with Immune Checkpoint Inhibitor-associated Nephritis

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Abstract Text: Immune checkpoint inhibitors inhibit the negative regulation of T cells, leading to both anti-tumor activity and immune-related adverse events such as acute interstitial nephritis (ICI-AIN). The immunologic drivers of ICI-AIN are unknown. We hypothesized that both resident and circulating $CD8^+$ T cell subsets are present in ICI-AIN and that they are conserved across kidney tissue and the urine sediment. To characterize the cellular populations that are enriched in ICI-AIN, we used scRNAseq to analyze epithelial and immune cells in kidney tissue and urine from a cohort of 27 patients with biopsy-proven ICI-AIN and 6 controls with non-AIN AKI. A total of ~150,000 cells were analyzed and revealed 24 transcriptionally distinct cell subsets, including $CD8^+$ and $CD4^+$ T cells, NK cells, B cells, myeloid cells, renal tubular epithelial cells, and urogenital epithelial cells. A range of T cell subsets was identified, existing on a phenotypic spectrum spanning circulation (KLF2) to residency (ITGAE), and cytotoxicity (IFNG, GZMK) to exhaustion (TIGIT). These cell subsets were conserved across both kidney tissue and urine. Gene expression analysis in ICI-AIN samples revealed populations of $CD8^+$ T cells expressing CXCR3 and myeloid cells expressing the interferon-y-stimulated ligands CXCL9/10, suggesting potential cell-cell interactions. Taken together, our data contribute to improved understanding of the cellular perturbations in ICI-AIN and reinforce a role for the IFN-y pathway in driving ICI-AIN. The conservation of T cell types observed across kidney tissue and urine suggests the potential for development of a non-invasive urine-based diagnostic test for ICI-AIN.

Th165. The ATP-binding Cassette Membrane Transporter MDR1 Promotes Tumor-specific CD8 T Cell Persistence

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Abstract Text: Multidrug resistance-1 (MDR1) is an ATP-binding cassette (ABC) membrane transporter known for effluxing chemotherapeutic drugs out of tumor cells. MDR1 is also expressed in normal cells, including immune cells, although its physiologic role is not well understood. MDR1 is dynamically expressed during CD8 T cell development and has been shown to promote survival of CD8 T cells responding to acute infection; however, little is known about the role of ABC transporters in tumor-specific CD8 T cells (TST). We previously developed a clinically-relevant genetic liver cancer mouse model in which adoptively transferred TST initially differentiate to a reversible dysfunctional state and later to an epigenetically-encoded fixed dysfunctional state driven by persistent tumor antigen stimulation. Surprisingly, late dysfunctional TST displayed the highest capacity to efflux ABC transporter substrates, greater than early dysfunctional TST or memory CD8 T cells. We similarly observed elevated efflux capacity in tumor-reactive CD8 T cells isolated from human hepatocellular carcinoma samples. RNA-sequencing confirmed increased expression of Mdr1a in late dysfunctional TST. We used CRISPR/Cas9 technology to generate MDR1-deficient TST, which displayed increased mitochondrial reactive oxygen species and persisted more poorly than MDR1-sufficient TST when adoptively transferred to tumor-bearing mice or exposed to cytotoxic chemotherapy. Our findings show that MDR1-mediated efflux is important for TST persistence and fitness, suggesting a role in mediating anti-tumor T cell responses in patients and regulating persistence following lymphodepletion. Future studies will explore the mechanisms underlying MDR1 function in CD8 T cells and test whether MDR1 modulation can improve anti-tumor adoptive T cell therapies.

Th166. The Human Commensal Bacterium, Erysipelatoclostridium Ramosum, Stimulates Cytotoxic CD8 T Cells and Overcomes Resistance to Cancer Immunotherapy

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Abstract Text: The gut microbiome impacts the efficacy of cancer immunotherapies in mouse models and patients. However, the mechanisms by which bacteria in the gut impact anti-tumor immunity in fighting tumors outside of the gut are unclear and microbiome-based therapies remain elusive. We have isolated a human commensal bacterium, Erysipelatoclostridium ramosum, that overcomes microbiome dependent resistance to immunotherapy. E. ramosum increases CD8 T cell proliferation in tumors and promotes anti-tumor responses in mice when orally administered live or dead. *In vitro*, co-culture of mouse or human CD8 T cells with dead E. ramosum, but not E. ramosum culture supernatant stimulates cytotoxic T cell proliferation and increases Granzyme B, perforin, and interferon gamma production suggesting that E. ramosum surface metabolites, and not secreted metabolites, stimulate cytotoxic T cells. To assess the specificity of E. ramosum for T cell stimulation, we tested 12 strains of human commensal bacteria in our co-culture system. While exposure to most bacteria trigger T cell death, E. ramosum can be cultured at doses up to 100 times higher than other commensal organisms and promotes cytotoxic CD8 T cells without inducing cell death. Furthermore, E. ramosum overcomes proliferation deficiencies induced by T cell proliferation inhibitors including p38, ERK, JNK, NFAT, JAK, PI3K, or AKT inhibitors. These results indicate that E.ramosum has a unique ability to stimulate CD8 T cells without damaging the immune response. Harnessing the mechanisms by which E. ramosum stimulates cytotoxic CD8 T cells could lead to more effective T cell-dependent cancer immunotherapies.

Th167. The Immune Regulatory Role of CEACAM6 in Human Pancreatic Adenocarcinoma

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Abstract Text: Immunotherapy has revolutionized cancer treatment in multiple tumors, but not in pancreatic ductal adenocarcinoma (PDA), one of the lethal malignant solid tumors. Carcinoembryonic antigen-related cell adhesion molecule (CEACAM6) is expressed 10-20-fold higher in PDA than normal pancreatic tissue. In PDA, CEACAM6 immunoinhibitory role remains unexplored. Therefore, we hypothesize that CEACAM6 regulates immune response in PDA by activating NF-□B. *In vitro* assays using spheroids and two-dimensional cultures of human PDA cells BXPC3 and HPAF treated with monoclonal anti CEACAM6 antibodies significantly restored $CD8^+$ T cell infiltration versus cell lines with untreated cell lines by almost two-folds ($p < 0.05$). T cell exhaustion markers are significantly lower post-anti-CEACAM6 treatment, accompanied by reduced $CD8^+$ T cell apoptosis 29% vs. 44% in HPAF-II treated vs. untreated, respectively. Amongst various cytokines tested, $TNF\alpha^+$ was significantly higher when co-incubated with T cells. We observed a significant reduction in NF-□B signaling in treated versus untreated by proteomic profiler and western blotting. We noticed an alteration in TLR4/MyD88/NF-□B axis in PDA cell lines upon activation with LPS, suggesting a potential collaboration between LPS-CEACAM6 in promoting immune evasion in PDA. Therapeutic targeting of CEACAM6 may be a promising strategy to improve PDA treatment and eliminate cell resistance to T cell cytotoxicity.

Th168. The Molecular Mechanisms of Treg Plasticity

Havva Marzban[1], Navid Saranjam[1], Aishwarya S Potturu[1], Abu Osman[1], Jasmin Quandt[2], Khashayarsha Khazaie[1] and Fotini Gounari[1]

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Abstract Text: The functional diversity of regulatory T cells (Tregs) is essential for maintaining healthy immunity but is also exploited in disease. We and others have causatively linked the gain of selective Treg proinflammatory functions to the expansion of a Rorγt^{+} CD4^{+} Foxp3^{+} Treg subset. We showed that in patients with inflammatory bowel disease (IBD) and colorectal cancer (CRC), as well as in the APCΔ468 murine polyposis model these cells overexpress β-catenin. Moreover, the expression of a dominant-active β-catenin in Tregs induces RORγt, leading to impaired suppressive function and the acquisition of Th17-like properties, ultimately resulting in a scurfy-like phenotype. Similarly, the loss of TCF-1 also upregulates RORγt but primarily affects the anti-inflammatory functions of Tregs without inducing the full spectrum of dysfunction observed with β-catenin activation. We showed that TCF-1 and HEB work together to regulate T cell development. In Tregs expressing dominant-active β-catenin, the deletion of either TCF-1 or HEB partially restores their function. However, the simultaneous deletion of both TCF-1 and HEB rescues Treg numbers and prevents the development of the scurfy-like phenotype. sc-RNAseq analysis revealed that HEB-deficient Tregs from mesenteric lymph nodes display an enhanced effector phenotype compared to WT and TCF-1KO Tregs, which may contribute to their functional restoration. Additionally, protein interaction predictions using the ARBD algorithm suggest a potential physical interaction between HEB and β-catenin. Collectively, these findings highlight a finely tuned collaboration among β-catenin, TCF-1, HEB, and RORγt to regulate Treg identity and function.

Th169. Understanding PD-L1 Regulation and Secretion: Insights to Improve Immune Checkpoint Inhibitor Therapies

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Abstract Text: PD-L1 Immune Checkpoint Inhibitors (ICI) have revolutionized cancer therapies, but less than 40% of patients benefit from them. Understanding the cell regulation of PD-L1 is key to explain the low response rates and improve therapy. PD-L1 is often expressed at the surface of tumor cells, particularly in the presence of IFNγ, leading to immune response inhibition. However, basal expression can vary from cell to cell. Importantly, PD-L1 can be released into circulation as a soluble protein or in extracellular vesicles (EV), both able to bind the PD-1 receptor. Despite their role in immune evasion, these soluble forms are often not considered in patient selection. Here, we compared the cell biology of PD-L1 in cancer cell transfectants with several bladder and metastatic melanoma cancer cell lines expressing endogenous PD-L1, where PD-L1 expression is typically much lower. A detailed analysis reveals that PD-L1 intracellular trafficking and secretion routes vary markedly between cancer cells from the same tissue, as does the intensity of IFNγ-induced PD-L1 upregulation. Notably, cells with high basal PD-L1 expression showed increased secretion of this molecule in EV. Furthermore, metalloprotease blockade can enhance PD-L1 secretion in EV, suggesting a connection between these two cellular pathways. Since secreted PD-L1 affects PD1 function, especially when multimerized in EV, variability in release routes may explain differences in ICI therapy outcomes. The intrinsic background of the cell, thus, affects multiple aspects of cellular PD-L1 expression. So, personalizing PD-L1 detection could be important to identify patients who would benefit from PD-L1 directed therapies.

Th196. Clonal Evolution Analysis of Paediatric Lymphoma Reveals a Highly Diversified Cellular Status and Potential Targets for Precision Medicine

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Abstract Text: Lymphoma is the third most common paediatric cancer. To reveal mechanisms underlying lymphomagenesis, we combined single cell RNA sequencing (scRNAseq) and immune receptor sequencing (VDJseq) of paediatric samples from non-cancerous reactive lymph nodes (n=7) and the three major types of paediatric lymphoma; Burkitt (BL, n=3), Hodgkin (HL, n=4), and T lymphoblastic lymphoma (TLL, n=5). VDJ clonal expansion was used for cancer cell annotation for BL and TLL. To investigate the tumour microenvironment in HL, we integrated scRNAseq with spatial transcriptomics. We identified a cycling cluster that consisted of dark-zone germinal centre B cells (DZGCBs) in reactive lymph nodes but harbours cancer cells from the BL and TLL samples, suggesting that the highly proliferative programme normally used by DZGCB cells is important for lymphomagenesis. Based on VDJseq, we identified hyperexpanded lymphoma clones in each BL and TLL. These lymphoma clones had a diverse transcriptomics profile, with BL sharing programme with germinal centre B cells, memory B cells, and plasma cells. TLL underwent a unique transcriptomic shift bridging the T helper cell cluster and cycling cluster, suggesting lymphomagenesis is continuous. Trajectory analysis showed a diverse transcription profile towards the cycling cluster, indicating the mechanism of this transition. Using a predictive treatment score, we observed heterogeneity in gene expression of the hyperexpanded clones highlighting the efficacy and short-coming of current treatment. Finally, spatial resolved transcriptomics revealed exhausted T cells proximal to Reed-Sternberg cells in HL. Our data reveals that paediatric lymphomagenesis is characterized by its diversified transcriptome and identifies novel targets for therapy.

Tu173. Advancing CAR T Clinical Development with High-Parameter CyTOF Technology

Lauren Tracey, Michael Cohen, Shakir Hasan, Stephen Li, Christina Loh and Ling Wang

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Abstract Text: Adoptive immunotherapy using chimeric antigen receptor (CAR) T cells is a revolutionary treatment in cancer therapy that has achieved great success in hematological B cell malignancies; however, it has faced significant challenges in solid tumors. A better understanding of CAR T biology will accelerate development of CAR T therapies with improved durability, antitumor efficacy and decreased toxicities. High-parameter cytometry is a powerful tool to functionally characterize CAR T cells at multiple stages of clinical development. CyTOF™ technology, or mass cytometry, overcomes the limitations that reduce the sensitivity of fluorescence-based cytometry, such as autofluorescence and signal overlap, and enables users to streamline workflows and minimize technical variations. Here, we present a 40-plus-marker CyTOF panel to simultaneously analyze protein expression of phenotypic and functional markers in single CAR T cells. CD19-targeted CAR T cells were expanded *in vitro* and co-cultured with Nalm6 cells for 2–5 days. Samples co-cultured at different time points were stained with a CyTOF panel including 40-plus surface, cytoplasmic and nuclear markers, barcoded, frozen and simultaneously acquired on a CyTOF XT system. Comprehensive profiling revealed that CAR T cells became activated, proliferated and produced cytokines in *in vitro* co-culture with tumor cells and showed reduced cytotoxicity and exhaustive phenotypes after an extended period of expansion. Overall, we demonstrate that CyTOF technology enables deep functional characterization of CAR T cells by simultaneous detection of surface, cytoplasmic and nuclear markers, supporting advances in CAR T therapy clinical development. For Research Use Only. Not for use in diagnostic procedures.

Tu175. Immunephenotyping of Tumor-infiltrating Immune Cells in the Microenvironment of Breast Cancer

Brian Silverio-Montes, Geobanni Torres-Montano, Alejandra Lopez-Vazquez and Carlos Velazquez-Contreras

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Abstract Text: Breast cancer is a heterogeneous disease where the tumor microenvironment (TMA), including immune infiltrates, plays a pivotal role in disease progression and therapeutic response. This study aimed to characterize immune cell phenotypes in the TMA across molecular subtypes of breast cancer. Formalin-fixed, paraffin-embedded central tumor tissues from 10 breast cancer patients (5 triple-negative (TN), 3 luminal B, and 2 $HER2^+$) were analyzed. Immunohistochemistry was performed for CD8, CD20, FOXP3, PD1, and HLA-DRα markers. Slides were digitized, and quantitative analysis was conducted using QuPath software (version 0.5.1). Cell segmentation and classification were achieved through a neural network, differentiating immune, fibroblast, and tumor cells. A stain intensity classifier provided percentages of positive cells for each marker. Preliminary results revealed distinct immune profiles among subtypes. TN tumors exhibited the highest HLA-DRα positivity (52.9%) and significant infiltration of $CD8^+$ (25.6%) and $PD1^+$ (15.3%) cells. Luminal B tumors showed elevated $CD8^+$ (32.7%) and $FOXP3^+$ (10.8%) expression but lower $PD1^+$ (3.6%) levels. $HER2^+$ tumors displayed notable $FOXP3^+$ (17.7%) positivity alongside reduced $CD8^+$ (14.9%) and $HLADRa^+$ (29.7%) expression. These findings highlight molecular subtype-dependent variations in immune cell infiltration. Elevated HLA-DRα in TN and higher FOXP3 in $HER2^+$ suggest differential immune modulation, potentially influencing prognosis and therapeutic strategies. Ongoing studies aim to validate these markers as predictive tools in the evolving era of immuno-oncology, with the goal of advancing personalized treatment strategies based on immune contexture.

Tu176. Impact of Incretin Mimetics on Circulating Immune Cells in Humans

Claire Pillsbury and Curtis Henry

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Abstract Text: In 2024, the weight loss drug class, GLP-1 receptor agonists (AKA: incretin mimetics or IM), accounted for up to 5% of all prescriptions written in the US. With indications for type 2 diabetes mellitus, obesity, obesity-related cardiovascular disease, sleep apnea, and other conditions, the application of these drugs alone or in combination is rapidly expanding. Obesity is an established risk factor for multiple cancers and induces immunosuppression by directly compromising the function of adaptive immune cells. However, it is currently unknown if the short- or long-term usage of IM impacts the function of immune cells in non-malignant settings or alters anti-tumor immunity. To determine any direct effects of IM on circulating immunity, we mined data from the Human Protein Atlas, which revealed that naive T cells and NK cells express genes coding for GLP-1 or GIP receptors. We performed flow cytometry on healthy human peripheral blood mononuclear cells, which demonstrated that a subset of circulating T cells expressed low levels of GLP-1R on their surface while monocytes expressed high levels of GCGR, both of which increased in samples from obese donors. Functionally, we also found that stimulating human T cells with the IMs semaglutide and tirzepatide significantly reduced the surface expression of the immunoinhibitory proteins TIM3 and LAG3. Based on these observations, and mounting evidence that IM may lower cancer risk, we will use mouse models of obesity and human samples to test our central hypothesis that IM will augment T cell function and enhance T cell-mediated elimination of cancer cells in settings of obesity.

W185. Myeloid-directed Innate Immunotherapy Enables Immune Rejection of Bladder Tumors

Sepideh Parvanian[1], Fan Fei[2], Ralph Weissleder[1] and Christopher Garris[1]

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Abstract Text: High-risk non-muscle invasive bladder cancer (NMIBC) is commonly treated with intravesical Bacille Calmette-Guérin (BCG) therapy. However, treatment failure and chronic BCG shortages leave many patients without effective options. To address this unmet need, we developed a triple-loaded nanoparticle system, CANDI, designed to enhance anti-tumor immunity through dendritic cell (DC) stimulation. CANDI incorporates R848, a TLR7/8 agonist, LCL-161, a cIAP inhibitor, and ruxolitinib, a JAK1/2 inhibitor, targeting canonical and non-canonical NF-κB pathways to upregulate IL-12 production. *In vitro* studies using bone marrow-derived dendritic cells (BMDCs) from IL-12 reporter mice confirmed that triple-loaded CANDI significantly boosts IL-12 production compared to dual-drug formulations, with enhanced activation of DCs. *In vivo*, CANDI demonstrated robust therapeutic efficacy in MB49 bladder cancer models. Mice treated with CANDI exhibited significant tumor size reduction, enhanced IL-12 production, and increased activation and trafficking of DCs to tumor-draining lymph nodes (tdLNs). This led to improved antigen presentation and T cell priming. CANDI was effective in both intravesical and intravenous administration routes, showing similar tumor targeting and immune cell activation. In a metastatic bladder cancer model, CANDI treatment reduced tumor burden, improved survival, and enhanced immune activation in the lungs and tdLNs. Mechanistic studies revealed that CANDI partially depends on $CD8^+$ T cells for efficacy and requires DC trafficking from lymph nodes to the tumor site. These findings highlight the potential of triple-loaded CANDI as a versatile therapeutic platform for both localized and metastatic bladder cancer, offering a promising alternative to current treatments like BCG.

W186. Optimizing CD45RA/CD45RO Co-staining on Human Peripheral Blood Samples

David Pillon, Derrick Lee, Michael Li, Takatoku Oida and Xifeng Yang

BioLegend, San Diego, CA

Abstract Text: CD45RA and CD45RO are widely used markers to differentiate between naive and activated/memory T cells in humans. Monoclonal antibodies HI100 and UCHL1 are two commonly used reagents to detect CD45RA and CD45RO, respectively. The change of CD45 variants expression during naive to memory T cell transition results in a distinctive "curved" staining profile of CD45RA and CD45RO in flow cytometric analysis. However, we observed that co-staining using HI100 and certain tandem dyes, such as PE/Cyanine7, conjugated UCHL1 on human whole blood samples can distort this characteristic “curved” profile. This phenomenon is donor-dependent and can be alleviated by analyzing pre-washed (or pre-lysed) whole blood or peripheral blood mononuclear cells (PBMCs). Sequential staining of UCHL1 followed by HI100 also overcomes the issue, while staining HI100 first did not. These findings suggest that plasma components might play a role in the interaction between HI100 and UCHL1 PE/Cyanine7 conjugates. Further investigation revealed that complement component 1q (C1q) was one of the key factors contributing to the atypical co-staining profile. Finally, we developed a novel anti-human CD45RA monoclonal antibody, clone S23030B, which does not exhibit this interaction with UCHL1 conjugates.

W187. Prognostic Value of Computerized Tumor-infiltrating Lymphocytes Analysis in Breast Cancer

Geobanni Torres-Montano[1], Brian Silverio-Montes[1], Alejandra Lopez-Vazquez[1], Adrian Daneri-Navarro[2] and Carlos Velazquez-Contreras[1]

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Abstract Text: Breast cancer remains one of the leading causes of cancer-related deaths globally, with tumor-infiltrating lymphocytes (TILs) playing a critical role in modulating tumor progression. In this study, we focused on the prognostic value of the eTILs% (the percentage of TILs in relation to the tumor cells) in a cohort of 94 breast cancer patients from Sonora, located in the northern region of Mexico. Patients were classified into three molecular subtypes: luminal (n = 61), HER2$^+$ (n = 20), and triple-negative (TN, n = 13). The study found that eTILs% showed a statistically significant correlation with disease-free survival (DFS) in luminal (cutoff = 3.97%, p = 0.048) and HER2$^+$ (cutoff = 13.45%, p = 0.023) subtypes. Specifically, lower eTILs% levels were associated with a higher risk of recurrence and death in these subtypes. However, in the TN subtype, due to the small sample size, no statistically significant cutoff value was identified, highlighting the need for further investigation with a larger cohort. Additionally, the analysis revealed higher lymphocytic infiltration in HER2$^+$ and TN subtypes compared to luminal tumors (p = 0.04 and p = 0.03, respectively). These findings suggest that eTILs% could be a potential prognostic biomarker, particularly for luminal and HER2$^+$ breast cancers, and that larger studies are necessary to validate its role in TN breast cancer. This study represents one of the first evaluations of eTILs% as a prognostic marker in a Sonoran population and provides valuable insight into the potential utility of immune response-based biomarkers in personalized cancer therapy.

W188. Quantitative Profiling of HLA Class I and II Antigens and Neoantigens in Tissue and PBMC Samples with an Optimized Mass Spectrometry Approach

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Abstract Text: Human leukocyte antigen (HLA) molecules are essential mediators of immune surveillance, presenting antigenic peptides to T cells and enabling immune recognition of pathogenic or abnormal cells, such as cancer. HLA-driven antigen presentation plays a central role in immunology and immuno-oncology, guiding immune responses and informing targeted interventions. In clinical trials, peripheral blood mononuclear cells (PBMCs) present a practical alternative to tissue biopsies for immunopeptidomics (IMPX) and neoantigen discovery. PBMCs are easily accessible via blood sampling, allow for serial monitoring, and encompass diverse immune cell populations, offering a comprehensive view of immune modulation and pharmacodynamics. We developed a high-sensitivity, robust workflow for profiling class I and II immunopeptidomes from PBMCs, tissues, and cell lines. Leveraging native lysis and magnetic bead-based immunoprecipitation, we achieved efficient extraction of HLA complexes from minimal starting materials, including as few as 5 million PBMCs. The pipeline integrates optimized LC-MS and AI-driven bioinformatics for reproducible and streamlined analysis. The workflow demonstrated high reproducibility and specificity across sample types, identifying robust immunopeptide profiles. >60% of class I immunopeptides were 9-mers, with >80% predicted as strong binders, showing expected amino acid enrichment at anchor residues. >50% of class II immunopeptides were 14-to-16-mers, with >50% predicted as strong binders. Input material ramping tests identified >2,800 class I immunopeptides from 2.5 mg of fresh-frozen tissue and 2,000–3,000 from 5 million PBMCs. In summary, we developed a high-sensitivity, robust pipeline for class I and II immunopeptidome profiling compatible with low-input samples, suitable for target discovery and clinical applications.

W105. Immune Remodeling of the Cancer Immunome After Chemotherapy and Adoptive Cell Transfer

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Abstract Text: Precision immunotherapy can potentially improve outcomes of cell therapy as a new frontier in cancer therapeutics. Understanding the effects of various therapies on the human immunome is crucial to enhance efficacy and reduce off-target effects. Adoptive immunotherapy with Epstein-Barr virus (EBV)-specific T cells (EBVST) has known clinical efficacy in EBV-associated nasopharyngeal carcinoma (NPC). As EBV ubiquitously infects humans, this facilitates comparison of both EBV-specific and systemic immune responses in healthy and diseased subjects. We compared global and specific immunity in age matched healthy controls versus NPC patients at baseline, and after standard combination chemotherapy, then sequential infusions of EBVST . Systemic immunity was greatly dysregulated in baseline NPC patients, who displayed reduced naive T cells and B cells compared to healthy controls. Conversely, baseline NPC patients had increased frequencies of T cells expressing the senescence marker CD57, and elevated monocytes and CD16+CD56dim NK cells. Chemotherapy and immunotherapy reduced the frequencies of circulating NK cells and monocytes, while chemotherapy in particular increased the frequency of effector CD8 T cells. EBV-specific responses were detectable in both healthy controls and NPC patients at all treatment timepoints investigated, though there was no significant difference in the frequencies of these subsets between groups. Our results corroborate previous findings of gross immune dysfunction in cancer, particularly involving depletion of naive lymphocytes. Future work will investigate functional differences in EBV-specific responses pre and post-immunotherapy and alterations in interactions between EBV-specific cell subsets and other immune subsets.

Infectious Diseases

Th170. Beyond Genetics: Lyme Disease's Unexpected Role in Connective Tissue Health - Findings from a Murine Model for Borrelia Burgdorferi Infection and the Human Clinical Study MAESTRO

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Abstract Text: Borrelia burgforferi (Bb) infection-associated chronic illness affects an estimated 2 million people in the US, and it is challenging to distinguish between immune vs pathogen mediated pathomechanisms. Label-free imaging of tissues from older (reproductively senescent) female C3H/HeJ mice infected long-term with Bb, revealed the presence of the bacteria in the connective tissues and indicators of pathology via gross anatomy and microscopic analysis. The use of a bioluminescent strain of Bb allowed for the identification of infected tissues *in vivo*, and these tissues were then imaged *ex vivo* via Label-free microscopy and orthogonally validated with histology. A hypermobile tail phenotype was observed to be infection status, age, strain, and sex specific, with distinct immune cell populations and features. Tensile testing revealed a strong inverse correlation between bacterial load and necessary force required to stretch the tissue. In our clinical study, MAESTRO, analysis of participants' previous medical history survey results identified a correlation between Bb infection-associated chronic illness and sequelae of hypermobility disorders in individuals who had Lyme disease, received antibiotics and never recovered. These data call into question a longstanding assumption that hypermobility disorders are in large-part benign and genetic, and suggest that previous Bb infection may play a role in the development of these diseases.

Th171. Lymph Node HIV-specific CD8 T Cells of HIV Controllers Harbour a Specific Transcriptomic Signature

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Abstract Text: Background. CD8 T cells have a preponderant role in controlling chronic infections. However, the precise mechanisms underlying the immune-mediated control of HIV replication remains to be identified. Lymph nodes (LN) represent a major site of viral persistence, but the study of anti-HIV CD8 T cells has been largely limited to peripheral blood. An in-depth characterization of LN HIV-specific CD8 T cells of HIV controllers may identify key determinants associated to viral suppression. Methods. We characterized blood and LN HIV-specific CD8 T cells from 5 chronic HIV infected individuals (CHI) and 4 HIV controllers by scRNA-seq and scTCR-seq. Longitudinal blood samples collected from 3 HIV controllers were also evaluated. Results. The cumulative data indicated that LN HIV-specific CD8 T cells of HIV controllers were enriched in a specific transcriptomic profile characterized by stemness markers (i.e. TCF7, IL7R, SELL), suggesting enhanced self-renewal capacity. This stem-like population in LN also expressed EBI2, responsible for T-B cell border positioning, and the effector molecule granzyme K. Canonical cytotoxic cells were enriched in blood but rare in LN in both CHI and HIV controllers. Longitudinal TCR sequencing revealed an evolution of TCR repertoire in some controllers, with expansion of formerly subdominant clonotypes. Conclusions. This study suggests that LN HIV-specific CD8 T cells in HIV controllers harbor a specific "stem-like" transcriptomic signature, possibly underlying an enhanced proliferative potential associated with an appropriate positioning. Interestingly, the TCR repertoire of HIV-specific CD8 T cells of HIV controllers can evolve with time, suggesting a dynamic adaptation of the immune response.

Th172. Role of Carbon Monoxide-producing Escherichia Coli Nissle 1917 in Reducing the Severity of Inflammatory and Infectious Diseases in Mice

Omar Vallejos, Pablo Reyes-Bustos, Guillermo Hoppe-Elsholz, Eduardo Tognarelli, Sofía Campos-Gajardo, Alejandro Piña-Iturbe, Irenice Coronado-Arrázola, Patricia Pereira-Sánchez, Pedro Silva, Francisco Quero, Valentina Scaff, Lucas Vásquez, Alexis Kalergis, Pablo González and Susan Bueno

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Abstract Text: Chronic infections and inflammatory diseases are a significant global health challenge due to rising prevalence and the limited efficacy of existing treatments. The enzyme heme oxygenase-1 (HO-1) in mammals has been widely recognized for its immunomodulatory effects, particularly through its byproducts like carbon monoxide (CO), which play key roles in controlling inflammation. However, no approved therapies currently target HO-1 activation. Probiotic bacteria have emerged as a promising approach for enhancing host responses to infections and inflammatory diseases. Among them, Escherichia coli Nissle 1917 (EcN), a well-studied probiotic with immunomodulatory properties, encodes a bacterial HO-1 homolog, ChuS, which contributes to heme utilization and CO production. In this study, we explored the role of ChuS in CO production and its impact on inflammation and infection. Using molecular genetics alongside *in vitro* and *in vivo* models, we demonstrated that ChuS activity enhances CO production in both settings and is essential for protection against DSS-induced colitis and herpes simplex virus type 1 (HSV-1) infection. Our results indicate that ChuS not only facilitates CO production but also reducing tissue damage and promoting host resilience during these conditions. These findings highlight ChuS as a key player in microbial CO production and suggest its therapeutic potential for modulating inflammatory responses. Using probiotics like EcN for CO delivery provides a solid basis for developing innovative therapeutic strategies targeting inflammatory and infectious diseases. This work was supported by FONDEF IDeA ID20I10082, Fondecyt Regular 1231905 and the Millennium Institute on Immunology and Immunotherapy (ICM-ANID ICN2021_045), a FOCIS Center of Excellence.

Th173. Systemic Cytokine Profiling of Pediatric Pulmonary and Extrapulmonary ARDS Reveals IL4 as a Common Putative Marker of Disease Severity

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Abstract Text: Introduction: Pediatric acute respiratory distress syndrome (PARDS) arises from direct insult to the lung parenchyma disrupting the alveolar epithelium [pulmonary PARDS (PARDSp)], or a systemic insult causing vascular endothelial damage [extrapulmonary PARDS (PARDSexp)]. This study profiles the circulating cytokines in PARDS to elucidate the underlying immunopathogenic differences between PARDSp and PARDSexp. Methods: Plasma from 24 patients (sepsis/PARDSexp, n=8 and pneumonia/PARDSp, n=16) and 8 controls (post elective cardiopulmonary bypass) were studied. The Olink® target 48 cytokine panel (Olink Proteomics) was used to quantify protein levels and reported as normalized protein expression. Principal component analysis (PCA) was used to visualise differences between groups. Multivariable linear regression was used to determine the association between PARDS severity and cytokine levels, adjusting for age and presence of sepsis. Correlation between cytokine levels and oxygenation index was performed using Pearson's correlation. Results: The mean (standard deviation) age of the cohort was 5.1(5.4) years. PCA plot readily distinguishes severe and non-severe PARDSp, PARDSexp and control. PARDSexp was associated with changes in 36/48(75.0%) systemic cytokines, whereas PARDSp was associated with only 22/48(45.8%). In the multivariable model, greater PARDS severity was associated with increased CCL8, CXCL9, IL4, IL18, CXCL10, IFNɣ, IL10, CCL19 and TNFα levels. Only IL4, IL18, CXCL10 and IFNɣ showed a progressive increase across the mild, moderate and severe PARDS where CCL8(r=0.35,p=0.047), IL4(0.82,p< 0.001) and CXCL10(r=0.42,p=0.016 had a positive correlation with oxygenation index. Conclusion: IL4, a cytokine reported to have anti-inflammatory effects and an inducer of trained immunity, was associated with PARDS severity in both PARDSp and PARDSexp.

Th174. The Aftermath of COVID-19 Epidemic-Are We on the Cusp of an Epidemic of Avascular Necrosis?

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Abstract Text: The COVID-19 pandemic was associated with the use of steroids at an unprecedented scale all over the world, and many patients required the use of intravenous bolus of methylprednisolone. High doses of steroids can be associated with avascular necrosis of the hips (AVN). A 38-year-old female presented to our center with progressive pain in the large joints. Her history was unremarkable except for a history of severe COVID pneumonia treated in the "second wave" with IV boluses of methylprednisolone two years earlier. Over the next few months, she had difficulty ambulating and had severe morning stiffness in the ankles, shoulders, and knees; the diagnosis was revised to rheumatoid arthritis since her rheumatoid factor and the ACPA were positive. She was initiated on DMARDs (arthritis medicines) and yet needed low-dose steroids and NSAIDs for symptom control, which now included stiff and painful shoulders and stiff ankles. There was a suboptimal response to DMARDs, and the patient continued to be miserable. The patient presented for a second opinion at our center after minimal response to treatment. The evaluation revealed AVN of the hips, shoulders, and talar domes. Her RF and ACPA were negative, and she had negative inflammatory markers. Her diagnosis was revised to osteonecrosis of multiple joints (AVN), and she was treated with NSAIDs and Bisphosphonates. Multi-site osteonecrosis is a rare entity and can mimic rheumatoid arthritis and spondyloarthritis; we may well be at the cusp of an epidemic of multisite AVN that will mimic rheumatoid arthritis over the next decade.

Th175. The Impact of Defective HIV Proviruses on Innate Immune-driven Inflammation

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Abstract Text: The persistent HIV reservoir includes a subset of cells harboring transcriptionally repressed latent HIV that contributes to rebound upon antiretroviral treatment (ART) interruption. Examination of proviral sequences shows the reservoir consists mostly of defective viral genomes with various mutations which would prevent production of HIV virions. People with HIV (PWH), even with suppression of viremia, demonstrate comorbidities of the central nervous system, heart, gut, and general aging-associated inflammation. These comorbidities are hypothesized to be driven by general inflammation and persistent circulation of pro-inflammatory cytokines. It is unclear if the latent HIV reservoir drives this inflammation. We previously demonstrated that transcription initiates from a promoter-like element located within HIV envelope gene. To investigate the impact of these transcripts, we employed CRISPR-cas9 to engineer cells harboring HIV genomes with a non-functional 5' Long Terminal Repeat (LTR), which acts as the enhancer and promoter for HIV proviral transcription. We observe a correlation between the levels of these internally driven transcripts with production of pro-inflammatory cytokines *in vitro* using macrophage-like cells (THP1), monocyte-derived-macrophages, and mononuclear cells obtained from PWH on ART. Knocking out MAVS abrogated this effect. We propose that defective HIV proviruses actively contribute to chronic inflammation in PWH via a MAVS-dependent pathway.

Tu177. The NLRC5/HLA-I Axis : A New Target to Elicit a Potent CD8$^+$ T Cell Response Towards Early HIV-infected Cells?

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Abstract Text: The absence of a cure or vaccine for HIV highlights the important need for innovative therapeutic strategies. Interestingly, rare patients called HIV controllers limit the viral replication with an effective immune response, including a CD8$^+$ T cell response targeting early infected CD4$^+$ T cells. This response is mediated by the recognition of viral antigens from incoming particles through HLA-I pathway. We hypothesize that enhancing the amount of presented viral antigens from incoming particles on infected CD4$^+$ T cells via upregulation of the HLA-I pathway could enhance the CD8$^+$ T cell response, thereby improving viral control. NLRC5, a key transactivator of the HLA-I pathway, is a promising candidate for this purpose. Notably, HIV proteins have been shown to downregulate NLRC5 expression in microglia, while epigenetic modifications of the NLRC5 gene have been observed in people living with HIV, potentially suggesting a viral immune evasion mechanism similar to that of SARS-CoV-2. Our preliminary data reveal that HIV infection induces epigenetic modifications of the NLRC5 gene in people living with HIV as well as transcriptomic and protein expression changes in CD4$^+$ T cells *in vitro*. We also developed tools to modulate NLRC5 expression in CD4$^+$ T cells and we are investigating its impact on CD8$^+$ T cell responses. These findings will then be confirmed *ex vivo* using cells from people living with HIV. By elucidating the role of NLRC5 in viral antigen presentation and CD8$^+$ T cell response, our study aims to advance novel strategies for enhancing immune control in HIV infection.

Tu178. The SPRED Study – Multiomic Prediction of Surgical Site Infections After Major Abdominal Surgery

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Abstract Text: Background: Postoperative complications, including surgical site infections (SSIs), occur in up to 44% of major surgeries, increasing mortality, hospital stays, and socioeconomic burden. Established clinical risk scores, such as the American College of Surgeons NSQIP (ACS) score, show limited predictive power. The SPRED study aimed to develop a predictive model for SSI after major abdominal surgery by integrating clinical and biological factors measured preoperatively [1]. Materials and Methods: In a five-center prospective study of 164 adult patients undergoing elective major abdominal surgery in France, SSIs were assessed within 30 days post-surgery. Preoperative clinical data and a mass cytometry and plasma proteomic (Olink) approach quantified 880 immune cell and 726 proteomic features in blood samples. Sparse machine learning and data-driven feature selection [2] using the Stabl framework were applied to build a multivariable SSI prediction model. Model performance was evaluated using Monte Carlo cross-validation (5-folds, 10 repeats) and compared to the ACS risk score [3]. Results: The integrative model combining clinical and proteomic data outperformed the ACS model (AUROC = 0.79 [0.70–0.88] vs. 0.50 [0.38–0.63]). Stabl identified three proteins (CXCL14, NCAM1, FABP6) and two clinical factors (ASA score, surgical indication) as key predictors. Conclusion: This integrative model provides superior accuracy in predicting SSIs compared to conventional factors, identifying biomarker candidates to personalize perioperative management.
[1] NCT05523713: Development and Validation of a Predictive Score for Surgical Site Infections (SPRED). [2] Hédou J et al. Nat Biotechnol (2024). https://doi.org/10.1038/s41587-023-02033-x [3] Bilimoria KY et al. J Am Coll Surg. 2013;217(5):833-42.e423. doi:10.1016/j.jamcollsurg.2013.07.385

Tu179. Type IV Pili and TLR2 Dependent Endocytosis of Episybiotic Saccharibacteria Modulates Epithelial Immunity

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Abstract Text: The human oral microbiome is a complex and dynamic community of microorganisms that play a crucial role in overall health by preventing pathogenic invasions and contributes to immune system training. Within few inflammatory diseases, recently discovered microbial dark matter, Saccharibacteria, increase their relative abundance. However, their role in these diseases are unclear. Additionally, Saccharibacteria are epibionts that live on the surface of Actinobacteria species and their interactions with the human host and inflammatory disease development are largely unexplored. We aimed to understand the unique immunological interactions of Nanosynbacter lyticus strain TM7x and its cognate host bacteria, Schaalia odontolytica strain XH001, with oral epithelial cells. Our findings demonstrated that TM7x dampen host and non-host bacteria induced innate immune responses. Metatranscriptomic analysis revealed several endocytic and signaling pathways induced by TM7x infection. Conversely, TM7x host bacteria induced TLR2 and NF-kB inflammatory cytokine pathways, while TM7x inhibited this pathway by directly interacting with the epithelial cells. The close interaction between TM7x and oral epithelial cells were mediated by Type IV pili TLR2 receptor interactions, respectively. Furthermore, TM7x clustered TLR2 receptor and endocytosed by the epithelial cells, inhibiting TLR2 activation by the host bacteria. Our studies demonstrate for the first time TM7x binding epithelial cell surfaces and being internalized into epithelial cells via caveolin mediated endocytosis. Upon internalization, TM7x survived within epithelial cells for 3 days before being eliminated by lysosomal degradation. Our results provide mechanistic insights into the largely unknown interactions between Saccharibacteria and their human host.

Tu180. Whole Blood Transcriptome Profiles Predict Worse Clinical Outcomes in Children Developing Acute Respiratory Distress Syndrome from Influenza Infection

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Abstract Text: Background: Influenza virus is a frequent cause of acute lower respiratory tract infection in children sometimes leading to life-threatening hypoxic respiratory failure called pediatric acute respiratory distress syndrome (PARDS). In children with PARDS, some will recover within weeks whereas others will have a prolonged clinical course that can lead to death. Immunologic predictors of more rapid recovery may lead insight into potential therapies. Methods: Children admitted to US pediatric intensive care units requiring invasive mechanical ventilation for influenza virus infection who met criteria for pediatric ARDS (n=69) were enrolled with parental consent. We compared bulk RNA-sequencing data from whole blood obtained from 39 (56.5%) pediatric patients who were liberated from invasive mechanical ventilator support within 14 days compared to 30 (43.5%) patients with more prolonged PARDS including 4 deaths. Differential gene expression analysis (fold change of at least 1.5 and FDR < 0.25) was performed using Partek Flow software. Results: Patients with prolonged recovery or death from influenza-related PARDS had significant enrichment of differentially expressed genes in pathways associated with neutrophil degranulation (corrected p< 0.01) including BPI, LCN2, MMP8, OLFM4, RETN, SERPINB10, TCN1, VAT1. Conclusions: These findings support the need for further investigation of the role of neutrophils and neutrophil extracellular traps in pediatric influenza-associated PARDS and death.

Tu181. A Humanized Mouse Model Reveals NETosis as a Key Driver of SARS-CoV-2-Induced Lung Pathology

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Abstract Text: Neutrophil extracellular trap (NET) formation, or NETosis, is a distinct response of human, but not mouse, neutrophils to SARS-CoV-2 infection. NETosis contributes to lung pathology and represents a potential therapeutic target. Here, we describe a novel humanized mouse model, MICTRG6, generated on the C57Bl/6N background, which enhances human myelopoiesis and supports the development of functional human neutrophils. Using *in vitro* and *in vivo* approaches, we demonstrate that NETosis in human neutrophils results from direct SARS-CoV-2 infection rather than phagocytosis and can be suppressed by inhibiting viral replication. Targeting key enzymes involved in histone citrullination or caspase-1 activity effectively blocked NETosis, implicating these pathways in disease progression. To mechanistically dissect the role of citrullination, we engineered humanized mice lacking PADI4, which exhibited significantly reduced NETosis in circulation and lungs during SARS-CoV-2 infection. Furthermore, treatment with Pefabloc, a serine protease inhibitor, effectively diminished NET formation, highlighting a promising therapeutic approach to mitigate lung pathology and inflammation. Notably, NETosis also influenced systemic immunity, as the elevated frequency of immature neutrophils in MICTRG6 mice—resembling clinical observations—was markedly reduced in the absence of NETosis. These findings provide key insights into the systemic impact of NETosis in SARS-CoV-2 infection and establish MICTRG6 as a valuable model for studying human neutrophil-driven pathophysiology.

Tu182. Amino Acid Metabolism Contribute to SIV Reservoir Size and Virus Rebound in Infants

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Abstract Text: Early-life immune environments differ from adults, shaping HIV infection and disease progression. Despite antiretroviral therapy (ART), inflammation persists, driven by soluble mediators that influence immune function, reservoir persistence, and rebound post-analytical treatment interruption (ATI). The relationship between metabolites, reservoir size, and viral rebound remains unclear. We investigated host- and microbial-derived metabolites in a pediatric SIV model. Eight infant rhesus macaques (RMs), 4 weeks-old, were orally infected with SIVmac251, with ART started 4 weeks post-infection. RMs underwent ATI after 60 weeks. Plasma samples were collected longitudinally for untargeted metabolomics (LC-MS) and plasma viral load measurements. SIV reservoir was evaluated at late ART. Modulation of metabolic pathways were validated using publicly available RNA transcriptomic dataset of SHIV-infected newborn and infant RMs. Lipid and amino acid metabolism in plasma were modulated longitudinally post-infection. These plasma metabolic profiles were also observed at transcriptional levels. Pre/early ART fatty acid and non-essential amino acid metabolism were negatively and positively associated with late ART reservoir size, respectively. Of note, amino acid metabolism during ART also contributed to reservoir maintenance and consequently to higher viral rebound post-ATI, while fatty acid metabolism showed inverse associations. This data indicates that the modulation of these pathways could modulate reservoir persistence. Amino acid metabolism can potentially promote viral persistence by supporting $CD4^+$ T cell activation and proliferation. Further validation of the direct role of these pathways in the immune system and in the reservoir maintenance are ongoing and could support new approaches for HIV cure.

Tu183. Bridging Studies with cGMP RBmHAXT(Δcys), a Tetravalent Vaccine for Lymphatic Filariasis, for Clinical Development

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Abstract Text: Lymphatic Filariasis (LF), a chronic parasitic infection caused by Wuchereria bancrofti, Brugia malayi, and Brugia timori, results in severe lymphatic dysfunction, leading to debilitating limb, breast, and genital edema. Although prophylactic administration of antiparasitic drugs has been used since 2000, disease transmission persists with irreversible lymphatic damage in patients globally. There is currently no vaccine available to protect the vulnerable population. Our laboratory previously developed a tetravalent vaccine, rBmHAXT(Δcys) that completed all pre-clinical studies, product development, and GMP manufacturing. Before initiating the clinical trials, there is a need to perform bridging experiments with the GMP rBmHAXT(Δcys) to confirm the vaccine efficacy and potency. Thus, in this study, we compared the immunogenicity and vaccine-induced protection of GMP rBmHAXT(Δcys) (three engineering runs) with our parent rBmHAXT(Δcys) protein in a mouse model. Our results show that the GMP rBmHAXT(Δcys) induced high titers (>1:40,000) of IgG antibodies and memory T cell responses to rBmHAXT(Δcys) that are comparable to the responses following immunization with the parent vaccine antigen. The vaccine-induced protection in GMP rBmHAXT(Δcys) immunized mice was (90.7 + 9.67%), which was similar to the protection seen in mice immunized with the parent protein (93.7 + 9.67%) and significant compared to control. These studies confirmed that the GMP protein retained the vaccine potential of the parent protein. These pre-IND-enabling studies will help bring the LF vaccine close to the clinics and aid in preventing the infection in 846 million people who are at risk of being infected in 52 endemic countries around the world.

Tu184. Duration and Mechanisms of Rhinovirus-mediated Viral Interference in Differentiated Human Bronchial Epithelium

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Abstract Text: Viral interference occurs when infection with one virus temporarily protects against infection with an antigenically distinct 2nd virus. Rhinovirus (RV) is the most common human respiratory virus and commonly co-circulates with other respiratory viruses, suggesting it may be a significant cause of viral interference. While animal models and *in vitro* work support the ability of RV to interfere with pathogenic respiratory viruses during acute infection, the duration of interference and specific mechanisms of protection are not well characterized. Here, we use a model of differentiated human bronchial epithelium to study acute and chronic transcriptomic and proteomic changes following RV infection. We find that RV infection induces a persistent antiviral state that leads to a 19-fold reduction in influenza A virus (IAV) replication upon challenge two weeks post-RV. This antiviral state persists after cessation of RV virus shedding and coincides with elevation of a small subset of interferon-stimulated genes (ISGs) and RV RNA. We find that protection against IAV does not require reinduction of a new interferon response upon challenge, suggesting that cells are in a long-term antiviral state likely mediated by direct antiviral effectors. Persistent ISGs in bronchial epithelial cells result from ongoing cytosolic dsRNA-sensing RLR signaling which can be reversed through inhibition of TBK1. Together, these findings show that RV induces a long-term antiviral state in airway epithelium through persisting RLR-signaling that protects against IAV through elevation of direct antiviral effectors.

Tu185. Ecoclusters: A Growing Catalog of Humanity's Collective T Cell Responses to Prevalent Immune Exposures

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Abstract Text: A subset of T cell receptors (TCRs) in a typical immune repertoire are present in many individuals exposed to the same immune exposure (e.g., virus). Previously (https://www.biorxiv.org/content/10.1101/2024.03.26.583354v1), we have used our database of tens of thousands of T cell repertoires to discover many groups of TCRs (called ECOclusters) that co-occur in the same people. We've shown that ECOclusters represent public T cell responses to a prevalent immune exposures by demonstrating that several ECOclusters comprise strong diagnostic markers for their associated exposures (SARS-CoV-2, CMV, EBV, HSV-1, HSV-2, Parvovirus and T. gondii). Here, we greatly expand our catalog of ECOcluster-disease associations in two ways: we've used our in-house MIRA assay to determine the antigen specificity of thousands of ECOcluster TCRs; and we've performed Adaptive Immunosequencing on hundreds of blood samples that have also undergone Infinity Bio's VirSIGHT assay, providing scores for the serological response of each donor to hundreds of viral pathogens. The result is a growing catalog of dozens of ECOclusters associated with the prevalent pathogens they respond to. These ECOclusters can provide diagnostic markers of exposure, and they can also describe the extent of a given individual's T cell response to a disease and their time since exposure. Further, substructure within the ECOcluster associated with EBV reveals different subgroups of people who respond in different ways, with some subclusters diminishing over time and others remaining throughout the person's life. ECOclusters are an increasingly powerful tool for describing a person's immune history and gaining insights into the different ways people respond to disease.

Tu186. Episodic Viremia in Women with HIV Alters IgG Galactose, IgG3 gp120, and Inflammatory Cytokines

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Abstract Text: People living with HIV (PLWH) often exhibit chronic low-level immune activation, contributing to increased susceptibility to chronic comorbidities. This process, called inflammaging, has been linked to IgG glycosylation patterns. It has been shown that a reduction in galactose modifications on IgG correlates with a pro-inflammatory state. Our study evaluates how episodic viral rebound affects IgG galactosylation and pro-inflammatory biomarkers, providing potential markers for HIV disease progression. Repository plasma from ten women with HIV (WWH) in the MWCCS (formerly WIHS) on cART, who experienced a single viremic episode (>200 copies/mL), was analyzed. Timepoints before, during, and after viremia were assessed. Subjects were stratified by viral load (low: < 1000 copies/mL vs. high: >5000 copies/mL). Bulk IgG galactose was quantified via ECL blot, HIV gp120-specific antibodies via ELISA, and inflammatory cytokines (e.g., IFN-y, IL-17a, IL-2) via Luminex. cART levels were measured by mass spectrometry. Six of ten WWH showed a 38% reduction in IgG galactose levels during viremia; four had a sustained reduction post-resolution (average 36% reduction). In high viral load cases, IgG3 gp120 inversely correlated with IgG galactosylation (p=0.0376), while IL-2 correlated positively (p=0.0378). IL-17a correlated with IgG3 gp120 across viral loads (p=0.0323). Eight of ten individuals had reduced or undetectable cART levels during viremia. These findings reveal IgG galactose reductions during viremia, indicating heightened inflammation and immune dysregulation. IgG galactosylation may serve as a valuable biomarker for inflammation and comorbidity risk in PLWH, highlighting the impact of episodic viremia on immune function and disease progression.

Tu187. Evaluation of the Vaccine Potential of a Cgmp Manufactured Vaccine for Clinical Development Against Lymphatic Filariasis

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Abstract Text: We have cGMP manufactured a tetravalent fusion protein vaccine for Lymphatic Filariasis (LF), a tropical parasitic infection caused by the parasitic worms Wuchereria bancrofti, Brugia malayi, and Brugia timori. The chronic infection causes severe lymphatic edema in dependent regions associated with marked lymphatic pathology. Lymphatic Filariasis (LF) is the second leading cause of disability in the world, leading to severe stigma in affected patients. There are no vaccines available to control this infection. About 886 million people worldwide need protection from this disease. A tetravalent vaccine, rBmHAXT (Δcys), developed in our laboratory has completed all pre-clinical studies, product development, and cGMP manufacturing. The focus of this study was to compare the immunogenicity and vaccine efficacy of the cGMP manufactured rBmHAXT (Δcys) with our parent rBmHAXT (Δcys) protein in a jird model. . Following infection, the parasite develops into male and female worms in jirds. We immunized jirds with 15 µg of rBmHAXT (Δcys) or the parent protein along with an adjuvant AL019 (alum+GLE). Our results show that all the immunized jirds developed high titers (>1:80,000) of IgG antibodies and antigen-specific T cells. The vaccine-induced protection in GMP rBmHAXT (Δcys) immunized mice was highly significant and comparable to the parent protein. Thus, the cGMP-manufactured rBmHAXT (Δcys) vaccine retained the immunogenicity and vaccine potential of the parent molecule. These studies will advance the LF vaccine to the clinic.

W140. Impaired STAT3 Signaling Disrupts Procalcitonin Production: Clinical Insights from Autosomal Dominant Hyper-IgE Syndrome

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Abstract Text: Procalcitonin (PCT) is an established biomarker for diagnosing bacterial infections and sepsis, yet the molecular mechanisms underlying its regulation are not fully elucidated. Recent studies highlight a central role for signal transducer and activator of transcription 3 (STAT3), a transcription factor activated by inflammatory cytokines, in mediating PCT synthesis. Autosomal Dominant Hyper-IgE Syndrome (AD-HIES or Job syndrome), a rare immunodeficiency characterized by dominant-negative STAT3 mutations, provides a unique clinical model to explore STAT3's role in human PCT responses. We describe two AD-HIES patients who developed severe bacterial infections yet exhibited paradoxically low serum PCT levels: Patient 1, a 22-year-old male, developed multifocal pneumonia with sputum cultures positive for Pseudomonas aeruginosa (PCT: 0.03 µg/L); Patient 2, a 17-year-old female, presented with methicillin-resistant Staphylococcus aureus bacteremia complicated by a hepatic abscess (PCT: 0.12 µg/L). Despite microbiological confirmation of bacterial infection and significantly elevated inflammatory markers (e.g., C-reactive protein), both patients exhibited markedly suppressed PCT responses. Our clinical observations align with prior mechanistic studies, supporting the essential role of STAT3-dependent signaling pathways in mediating PCT synthesis in response to bacterial-induced cytokines and endotoxins. While experimental models have demonstrated that reduced STAT3 activity decreases PCT expression, our findings uniquely illustrate this relationship through naturally occurring STAT3 impairment in AD-HIES patients. These cases deepen the mechanistic understanding of PCT regulation and underscore the importance of carefully interpreting serum PCT levels in immunodeficient patients, advocating for personalized approaches in infection diagnosis and management.

W189. Exploring the Role of Monoclonal Antibody Therapy and Lipid Metabolic Alteration in Human Respiratory Syncytial Virus and Herpes Simplex Virus Type 1

Mónica Farías[1], Catalina Andrade[1], Felipe Cancino[1], Areli Navarro[1], Ricardo Loaiza[1], Linmar Rodríguez-Guilarte[1], José Muñoz[1], Luisa Duarte[2], Ignacio Pastén-Ferrada[1], Abel Soto[1], Eduardo Tognarelli[1], Almendra Castillo[1], Maximiliano Ramm[3], Barbara Alarcón[3], José Cordero[1], Sergio San Martín[3], Natalia Muñoz-Durango[1], Magdalena Pizarro-Ortega[1], Benjamín Diethelm-Varela[1], Alejandra Pereira-Serrano[1], Robinson Ramírez[1], José Chávez[1], Cristian Agurto-Muñoz[3], Angello Retamal-Díaz[4], Nelson Barrera[1], Luis Bustamante[3], Karen Bohmwald[5], Jorge Soto[6], Susan Bueno[1], Pablo González[1] and Alexis Kalergis[1]

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Abstract Text: Human respiratory syncytial virus (hRSV) and herpes simplex virus type 1 (HSV-1) are prevalent pathogens with significant morbidity. hRSV causes acute lower respiratory infections in infants, while HSV-1 establishes lifelong infections through neuronal latency. Both viruses lack approved vaccines for at-risk populations, emphasizing the need for innovative therapeutic approaches. This study explores a monoclonal antibody (mAb) therapy against hRSV and the role of lipid metabolism in the pathogenesis of both viruses. We developed four high-affinity humanized anti-N-hRSV mAb clones and tested them in a BALB/c murine model. Treatment with anti-N-hRSV mAb reduced pulmonary and long-term neurological pathologies associated with hRSV. To understand the mechanism of long-term neurological pathologies, we also examined the hRSV impact on central nervous system (CNS) cells, focusing on neutral lipid metabolism, which is linked to neurodegenerative pathologies. *In vitro* studies

revealed that hRSV alters the neutral lipid composition of CNS cells and their lipid gene expression. Likewise, we have found that HSV-1, a neurotropic virus, disrupts lipid metabolism in neuronal cells, inducing lipid droplet (LD) formation. Inhibiting lipid uptake reduced HSV-1 propagation and restored MHC-I expression. These findings align with our dendritic cell (DC) studies, where HSV-1 infection reduced lipid catabolism and increased lipid synthesis pathways, promoting triglyceride and ceramide accumulation, which impaired DC function. Pharmacological inhibition of lipid synthesis pathways in HSV-1-infected DCs reduced LD accumulation and viral replication, improving DC viability and immune response. These results suggest that anti-N-hRSV mAb and lipid metabolism may be promising targets for antiviral therapies against hRSV and HSV-1.

W190. Expression, Purification, and Evaluation of a Novel Tetravalent Vaccine Antigen for Its Vaccine Potential Against Heartworm Infection

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Abstract Text: Heartworm infections in canines and felines cause severe weight loss, exercise intolerance, cough, and labored breathing, leading to heart and kidney failure. The disease is caused by Dirofilaria immitis, a filarial nematode parasite, and is transmitted by mosquitoes. Currently, there are no vaccines available to control the infection. In this study, we selected four genes of D. immitis, based on their reactivity to immune dog sera and previous published reports and their homology to vaccine antigens reported from other closely related filarial parasites. The recombinant tetravalent protein was commercially synthesized at Genscript. After removing the endotoxin from the preparation, mice (n=10) were immunized three times at two-week intervals with 15 µg of the recombinant antigen and 100 µg of AL019 adjuvant (Alum + GLE, a TLR-4 agonist). Titer of IgG was measured in the sera of immunized mice after each immunization and showed significantly (P< 0001) high titer compared to the control. All mice were challenged with 10 L3 larvae of D. immitis and the results are awaited. Thus, our studies identified four potential vaccine antigens that were developed as tetravalent vaccines that are highly immunogenic. This vaccine can be further developed as a prophylactic vaccine against heartworm infection.

W191. Increased Shedding of the interleukin-6 Receptor Contributes to Reduced Vaccine Efficacy in Obesity

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Abstract Text: Obesity is associated with increased mortality from COVID-19. Our previous work showed vaccines against this virus are less effective in people with obesity due to the rapid waning of vaccine-induced antibody titres. Disrupted IL-6 signalling may contribute to this state of relative immunodeficiency in obesity. Using single-cell RNA sequencing of SARS CoV-2 Spike-protein specific B lymphocytes, we found that people with severe obesity exhibit a reduction in antibody class-switched memory B cells 28 days after COVID-19 vaccination. In addition, we found using CITE-seq that IL-6R expression is reduced on B cells in patients with severe obesity. Flow cytometry validated reduced IL-6R surface expression on all lymphocytes, and impaired STAT3 phosphorylation was seen in response to IL-6 stimulation. In IL-6R deficient mice, vaccine-induced antibody titres declined more rapidly, mirroring our human data. In severe obesity circulating soluble IL-6R levels were elevated, indicative of increased shedding of the receptor. A common non-synonymous single nucleotide polymorphism in the IL6R gene, Asp358Ala (rs2228145), causes increased proteolytic ectodomain shedding, and reduced surface IL-6R expression. SNP genotyping of our cohort demonstrated that carriage of this common variant was associated with reduced surface IL-6R expression,

but that obesity also leads to a further marked reduction in expression, regardless of genotype, compared to normal weight counterparts. In conclusion, these findings suggest intersecting effects of genetics and BMI leading to reduced surface expression of IL-6R, impaired IL-6-mediated B cell differentiation and reduced vaccine efficacy. Selective enhancement of IL-6R signalling during vaccination may improve vaccine effectiveness in people with obesity.

W192. Maturation of the Cytokine Response to Sequential Plasmodium Falciparum Challenges

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Abstract Text: The development of natural immunity against malaria requires multiple exposures and even after protection develops against clinical disease individuals continue to be susceptible to infections. To study the dynamics of the immune response to repetitive P. falciparum-infected mosquito exposures, we used controlled human malaria challenges (CHMI) with the same strain of P. falciparum. Consistent with the gradual immune response observed in the field, after 4 sequential parasite exposures over 2 years, symptoms decreased and the time to parasite detection on a blood smear increased significantly, but all the volunteers continued to be susceptible to infections. Anti-parasite antibody levels varied between individuals, but overall gradually increased through the repeat exposures. Cytokine responses peaked with peak parasitemia, except for IL-18 which continued to increase for another 7 days. Despite decreasing symptoms after repeat exposures, cytokine levels remained similar except IFNγ, which could be due to the decrease in γδ2 or increase in γδ1 T cells observed after repeat exposures. The cytokine profile was compared to that observed in a cohort of pediatric malaria patients aged 1-13 years. As in the CHMI data, the cytokine profile was similar for 1-2 year-olds and those >2 years which is consistent with exposure not decreasing the response. IL-18 was the one exception and it was higher in younger children. Levels of most of the 23 cytokines tested were comparable between the children and CHMI participants. However, IL-10 and IL-8 were markedly higher in children, while IL-1RA, IL-21, IL-4 CCL4, CCL3 were higher in the CHMI participants.

W193. Nasal Biomarker Testing to Rule out Viral Respiratory Infection and Triage Samples: A Test Performance Study

Julien Amat, Sarah Dudgeon, Timothy Watkins, Nagarjuna Cheemarla, Alex Green, Patrick Young, David Peaper, Marie Landry, Wade Schulz and Ellen Foxman

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Abstract Text: The COVID-19 pandemic revealed an urgent need for practical screening tests to rule out respiratory infections for managing outbreaks and for routine screening in high-risk settings. PCR is the gold standard test for respiratory virus diagnosis but requires specialized equipment, is pathogen-specific, and may not be immediately available for an emerging virus. This study evaluated the performance of nasopharyngeal CXCL10, a host biomarker of diverse viral respiratory infections, as a virus-agnostic screening test to rule out viral respiratory infection. We also evaluated how viral prevalence and patient clinical features affect test performance. We compared CXCL10 immunoassay to comprehensive respiratory virus PCR test results from 1088 nasopharyngeal samples. We used mathematical modeling to determine the negative predictive value (NPV) of CXCL10, and the prospective impact on patient triage and resource savings across varied viral prevalences, guided by real-world data from the COVID-19 pandemic. We used machine learning-based outlier analysis to explore clinical features associated with false negatives. In our sample set, 32.6% of samples were virus-positive by PCR, and CXCL10 concentration accurately predicted virus positivity (A.U.C. 0.87, 95%CI 0.85-0.90). Model predicted that CXCL10 screening would enable a significant reduction in PCR testing, especially when viral prevalence is low (e.g., 92% of samples testing negative when viral prevalence is 5%). Outlier analysis identified features associated with false negatives including specific

chemotherapeutic drugs and low viral load. These results demonstrate the utility of a nasopharyngeal biomarker to rule out respiratory infection, with potential applications in outbreak management and/or routine screening in high-risk settings.

W195. Single-cell Multi-omic Profiling of Human Influenza Challenge Uncovers Dynamic Peripheral Innate Responses Modulated by Disease Severity and Viral Shedding

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Abstract Text: Innate immune cells play a crucial role in early antiviral defenses, mediating initial interferon (IFN) responses for pathogen clearance, and may exhibit trained immunity through lasting epigenomic changes. We hypothesized that disease severity and shedding status may modulate the magnitude of acute and long-term innate responses. To test this, we performed paired single-cell RNA and ATAC sequencing on peripheral blood samples from a cohort of 12 healthy adults challenged with influenza (H1N1), and measured pre-infection, acute stage, and one-month post-infection timepoints. We found that people who developed an infection had reduced proportions of NK cells at baseline compared to non-shedders. During acute infection, we found that IFN-stimulated genes (ISG) responses in $CD14^+$ and $CD16^+$ monocytes positively correlated with viral shedding. Notably, ISG responses were negatively correlated with disease severity at day 3 and shifted to a positive correlation by day 6. Additionally, we found that there was a day 6 emergence of intermediate monocyte-like cells across all subjects with symptomatic infections. Further, we updated our single-cell analysis method, called single-cell MetaIntegrator, to analyze paired single-cell ATAC-seq data. Using this new method, we show that one-month post-infection, $CD14^+$ monocytes of viral shedders showed persistent changes, including upregulated immune activation genes and significant differences in chromatin accessibility compared to baseline, while asymptomatic non-shedders showed minimal to no differences. In summary, our findings emphasize the importance of coordinated early IFN responses in managing disease severity and suggest that symptomatic influenza may persistent epigenetic changes, offering new insights into disease.

Mucosal Immunology

Th176. EML4 Deficiency as a Novel Cause of Alveolar Macrophage Dysfunction and Interstitial Lung Disease

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Abstract Text: Alveolar macrophages (AMs) play a critical role in maintaining lung homeostasis. Thus, identifying regulators of their function is important to understanding the pathogenesis of lung disease. Two siblings born to consanguineous parents presented with early onset progressive interstitial lung disease and a pulmonary alveolar proteinosis (PAP)-like phenotype, as well as failure to thrive and atopy. We identified homozygous deletions in the EML4 gene, encoding the Echinoderm microtubule-associated protein-like 4 (EML4), in both children. EML4 deficiency has not been reported previously, and there is limited literature regarding its function. Thus, we generated mice with a similar deletion in Eml4 to examine the role of EML4 in lung immunology and to understand the clinical features of the patients. Analysis of EML4-deficient mouse lungs revealed a decrease in AMs, with scRNA-seq demonstrating the most differentially expressed genes in this population, compared to WT mice. AMs from EML4-

deficient mice also had increased lipid content, consistent with the foamy macrophages in the patients' lungs. EML4-deficient mice did not demonstrate lung pathology under pathogen free conditions. However, exposure to influenza virus or a more diverse microbiota by co-housing with 'dirty' mice led to significant lung damage and immune infiltration EML4-deficient mice. Importantly, bone marrow transplant from WT mice restored AM numbers in EML4-deficient mice, with scRNA-seq demonstrating a correction of the AM phenotype. This suggests a possible treatment option for affected patients. Thus, we report the first cases of human complete EML4 deficiency and identify EML4 as a critical regulator of AM function and lung homeostasis.

Th178. TGF-β3 Induces Oral Tolerance by Imprinting Gut-Homing Capacity in Regulatory T Cells

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Abstract Text: Immune responses against ingested proteins are avoided by a poorly understood mechanism known as oral tolerance (OT). In a previous study, we demonstrated that OT depends on TGF-β3 but occurs in the absence of TGF-β1. Here, we sought the cellular source of TFG-β3 during OT and analyzed the mechanism through which it induces the process. We used conditional knockout mice (cKO) to identify the cellular lineages that produce TGF-β3 during OT. We used ovalbumin (OVA) as a model antigen and OT-II FoxP3GFP cells to analyze Treg generation. OT was tested in a model of delayed-type hypersensitivity and anti-OVA antibodies were quantified. Conditional deletion of Tgfb3 in all cell lineages (Tamoxifen-Cre) abolished OT and enhanced anti-OVA antibody production. Deletion of Tgfb3 in hematopoietic cells (iVav-Cre) significantly impaired OT. Deletion of Tgfb3 in CD11c^{+}, TCR-αβ^{+} (CD4-Cre), and TCR-γδ^{+} cells had no effect. Transcriptomic comparison of CD4 cells activated with TGF-β1 vs. TGF-β3 revealed that TGF-β3 promotes the expression of gut-homing molecules. This was confirmed using adoptively transferred OT-II cells, as OVA-specific Tregs were significantly less abundant in the small intestine lamina propria (siLP) in Tgfb3 cKO mice and expressed lower levels of CD103 and α4β7 integrin. Accordingly, during steady state, lack of TGF-β3 decreased the abundance of siLP Tregs and their expression levels of FoxP3, CD103, and α4β7 integrin. In conclusion, TGF-β3 produced mainly by hematopoietic cells is essential for oral tolerance. This cytokine imprints Tregs with an adhesion molecule expression profile necessary for their correct functionality in the gut.

Tu188. Alterations of the Nasal and Oral Microbiota in Multiple Sclerosis

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Abstract Text: While changes in the gut microbiota have been identified in multiple sclerosis (MS), little is known about the nasal and oral microbiota, which are important components of the mucosal immune system. The gingival microbiota has been shown to drive systemic inflammation, the pharyngeal microbiota can lead to autoimmune-mediated neurologic diseases, and altered bacterial toxigenic genes in the nasal microbiota have been found in active MS. We investigated nasal, gingival, and oropharyngeal microbiota from MS and healthy control subjects. We profiled the microbial composition using 16S rRNA sequencing and identified site-specific microbiota differences in both the nasal and oral microbiota related to relapsing and progressive MS. We identified several opportunistic bacteria that were associated with disease worsening in MS using linear regression analysis while controlling for

confounding factors and subject demographics. Using baseline microbiota samples and clinical outcomes over time, we identified potential beneficial microbes, including *D. pigrum* and *Corynebacterium sp*. in the nasal microbiota and *Prevotella sp*. in the oral microbiota, as well as potential detrimental *Streptococcus sp*. in the oral microbiota. Taken together, our data suggests that nasal and oral microbiota are altered in MS, are linked to the disease course and provide an avenue to better understand MS pathogenesis and treatment.

Tu189. Bronchoalveolar and Airway Mucosal T Cell Phenotypes and Repertoires Are Distinct in Health and Allergic Asthma

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Abstract Text: Asthma affects ~8.7% of individuals in the United States and most commonly is associated with Type 2 inflammation. T cells, specifically T helper type 2 (Th2) cells, drive allergic asthma. Previous studies have examined T cells from the lungs of asthmatics captured via bronchoalveolar lavage (BAL), endobronchial brushings or biopsies. However, whether these different approaches are capturing the same T cell phenotypes and/or clonotypes is unknown. To evaluate this, single-cell RNA sequencing was performed on $CD3^+$ T cells isolated from paired BAL and endobronchial brushings in 3 healthy controls (HC) and 3 allergic asthmatics (AA). T cell receptor (TCR) sequences were recovered using TRUST4 software. Unbiased clustering allowed us to define 6 CD4 and 7 CD8 T cell subsets. Interestingly, Th2 cells were enriched in AA compared to HC when comparing endobronchial brush samples (OR=26.2, P=0.002), but not when examining BAL (OR=1.7, P=0.46), indicating differences in the T cells isolated from the BAL and airway mucosa. In further support of this concept, an up-regulation of resident-memory T cell genes (i.e. ITGAE, CD69) was observed broadly in the cells from endobronchial brushes compared to BAL across T cell types of both lineages. Lastly, TCR repertoire analysis from AA and HC revealed expanded clones with Th1 and cytotoxic CD8 T cells phenotypes found exclusively in the airway mucosa. These results demonstrate that T cell phenotypes and clonotypes are distinct in bronchoalveolar and airway mucosa compartments and sampling the airway mucosa is critical to define T cell biology in diseases such as asthma.

Tu190. Dietary Fibre Counters the Oncogenic Potential of Colibactin-producing Escherichia coli in Colorectal Cancer

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Abstract Text: Diet, microbiome, inflammation and host genetics have been linked to colorectal cancer (CRC) development, however it is not clear whether and how these factors interact to promote carcinogenesis. Here, we used $Il10^{-/-}$ mice colonized with bacteria previously associated with CRC: enterotoxigenic Bacteroides fragilis, Helicobacter hepaticus, or colibactin-producing (pks^+) Escherichia coli and fed either a low carbohydrate (LC) deficient in soluble fibre, a high fat-high sugar, or a normal chow diet. Colonic polyposis was increased in mice colonized with pks^+ E. coli and fed the LC diet, that was dependent on colibactin. Mechanistically, mucosal inflammation was increased in the LC diet-fed mice, leading to diminished colonic PPAR-γ signalling and increased luminal nitrate levels. This promoted both pks^+ E. coli growth and colibactin-induced DNA damage. PPAR-γ agonists or supplementation with dietary soluble fibre in the form of inulin reverted inflammatory and polyposis phenotypes. Since colibactin toxin produced by pks^+ E. coli causes DNA adducts, interstrand DNA cross-links and double-stranded DNA breaks (DSBs), we also tested whether deficiencies in DNA repair pathways that repair DNA lesions

produced by colibactin could increase susceptibility to colon carcinogenesis. Indeed, pks$^+$ E. coli induced more polyps in mismatch repair (MMR)- deficient mice by inducing a senescence-associated secretory phenotype. Moreover, oncogenic effects were further potentiated by inflammatory triggers in the MMR-deficient model. These data reveal a dynamic interplay between diet and host genetics that shape the local mucosal milieu to influence the oncogenic potential of a common pathobiont.

W196. Atlas of Promoter-enhancer Interactions in Human ilc3s Links Crohn's Disease-associated Variants with Established and Newly Implicated Target Genes

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Abstract Text: Type 3 innate lymphoid cells (ILC3s) are tissue-resident lymphocytes that maintain homeostasis and regulate inflammation at mucosal barriers. To gain insight into the cis-regulatory circuitries underlying ILC3 function, we used high-resolution Promotor Capture Hi-C to map interactions between promotors and distal regulatory elements in primary human ILC3s. We found that promoter-interacting regions in ILC3s are enriched for genetic variants associated with multiple autoimmune diseases. Given the established role of ILC3s in Crohn's disease, we devised a Bayesian approach incorporating multivariate fine-mapping to link Crohn's-associated genetic variants with their putative target genes. This algorithm identified 33 previously known and 56 newly implicated genes expressed by human ILC3s that increase risk for Crohn's Disease. One noteworthy candidate gene, Cln3, encodes a ubiquitously expressed transmembrane lysosomal protein. Loss-of-function mutations in Cln3 cause the pediatric neurodegenerative disorder Batten disease. Although extensive research has explored the role of Cln3 in the nervous system, our study is the first to implicate Cln3 in an immune-mediated disease. Therefore, we aimed to experimentally define the ILC3-specific functions of Cln3 and uncover the mechanisms by which Cln3 dysfunction increases Crohn's Disease risk. We developed CRISPR-dCas9 interference and activation systems in mouse ILC3s to precisely modulate expression of Cln3. Functional studies suggest that Cln3 maintains ILC3 homeostasis through regulating autophagy, managing oxidative stress, and modulating inflammatory pathways. Our ongoing research aims to rigorously define the function Cln3 in ILC3 biology, with the potential to reveal common mechanisms underpinning diverse neurodegenerative and inflammatory diseases.

W197. Enhanced Clonality and Autoantibody Production by Antibody-Secreting Cells in CRSwNP

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Abstract Text: Background: Nasal polyps (NP) in chronic rhinosinusitis with NP (CRSwNP) contain increased extrafollicularly activated antibody-secreting cells (ASCs) that produce autoantibodies, including anti-dsDNA IgG, which predict NP recurrence post-surgery. However, the cellular profile and antigen receptor repertoire of ASCs in NP remain unclear. Methods: NP, tonsil tissues, and blood were collected during surgery. ASCs, including plasmablasts (PB, CD38$^+$CD138-) and plasma cells (PC, CD38$^+$CD138$^+$), as well as IgG$^+$ plasma cells, was quantified by flow cytometry. B and T cell receptor repertoires (BCR and TCR) were assessed using clonality (mean frequency of unique CDR3 sequences) and Diversity Index 50 (DI50), representing clones making up 50% of the total repertoire. Results: In NP tissue (n=5), PB frequency was significantly lower than in tonsils (n=6) ($p< 0.05$) but comparable to blood (n=4). Conversely, PC frequency was significantly higher in NP than in blood ($p< 0.05$) and tonsils ($p< 0.01$). BCR-seq indicated increased clonality and lower DI50 in NP, and IGHV3-34, a dsDNA-reactive Ig heavy chain, while TCRβ-seq showed marginal differences. Additionally, recurrent NP exhibited a trend toward lower DI50 versus non-recurrent NP. Conclusions: Extrafollicularly activated ASCs in NP tissue predominantly consist of

PCs expressing the mature B cell marker CD138. B cells in NP display increased BCR clonality, particularly in recurrent NP.

W198. Pooled Faecal Microbiotherapy Can Curb Refractory Gastrointestinal Acute Graft-versus-Host Disease Likely Through DP8a Treg Induction/Activation

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Abstract Text: The conditioning regimen preceding allogeneic hematopoietic stem cell transplantation leads to a significant gut microbiota dysbiosis, characterized by a reduced diversity and an altered microbial composition. This dysbiosis promotes Graft-versus-Host Disease (GvHD) severity and mortality in both mice and humans. Therefore, restoring a healthy and diverse gut microbiota offers a promising strategy for GvHD treatment. MaaT Pharma developed MaaT013, a microbiotherapy derived from pooled healthy human faecal microbiota, with high bacterial diversity. In a Phase III clinical trial, MaaT013 demonstrated a 62% gastrointestinal overall response rate and a 54% 1-year survival probability in refractory gastrointestinal acute GvHD (GI-aGvHD) patients. To evaluate MaaT013's impact on patients' immune system, blood samples (14 patients) were collected from the ancillary ORION study to the expanded access program (NCT04768907) evaluating MaaT013 in refractory GI-aGvHD. We characterized DP8a Tregs, a Treg subset identified by our laboratory in the human colon and blood, TCR-specific for *Faecalibacterium duncaniae*, a Firmicutes member, highly represented in MaaT013. Remarkably, DP8a Tregs expanded only in MaaT013-responding patients, suggesting that MaaT013 could stimulate DP8a Tregs to curb GI-aGvHD. Additional investigations revealed that DP8a Tregs can cross-react with Akkermansia muciniphila, another bacterium contained in MaaT013. These cross-reactions, and potentially others, could explain the DP8a Treg-mediated anti-inflammatory effect observed both *in vitro* and *in vivo*. We further demonstrated that MaaT013 induces anti-inflammatory and Treg-polarizing molecules on dendritic cells, likely contributing to the induction/activation of DP8a Tregs. Altogether, these data suggest the key role of DP8a Tregs to alleviate GI-aGvHD in response to MaaT013 faecal microbiotherapy.

W220. The Ulcerative Colitis Susceptibility Gene Adenylyl Cyclase 7 Restrains Th2 Cytokine Production and Class II Antigen Presentation

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Abstract Text: Genome-wide association studies have shown that the most risk-conferring genetic polymorphism for ulcerative colitis outside the human leukocyte antigen locus is the amino acid substitution p.Asp439Glu in the adenylyl cyclase 7 gene (ADCY7). ADCY7 is the main isoform in the hematopoietic system and produces the second messenger cyclic AMP (cAMP) downstream of G protein-coupled receptor signaling. Our aim was to determine the contribution of this polymorphism to UC risk by analyzing its effect on ADCY7 function in cell-based assays. We characterized the p.Asp439Glu variant in cell lines using western blots, immunofluorescence, cAMP assay, and luciferase assay. We modeled this variant using siRNA knock-down in human primary CD4+ T cells and characterized them by RNA-seq, viability assay, flow cytometry, cAMP assay, and ELISA. The p.Asp439Glu variant is deficient in protein expression but retains membrane localization. This results in a 40% reduction in cAMP

synthesis and luciferase reporter expression. Knock-down of ADCY7 in T cells reduces the expression of ribosomal proteins and cAMP signaling proteins, while skewing cytokine production towards a Th2 pattern. ADCY7 knock-down further upregulated antigen presentation, accompanied by increased cell surface expression of MHC Class II and CD86. The ulcerative colitis risk-conferring variant, p.Asp439Glu, in ADCY7 reduces cyclic AMP signaling, leading to modifications in cytokine profile and antigen presentation. Medications that enhance cyclic AMP by direct activation of ADCY7 or by phosphodiesterase inhibition may be beneficial in this disease.

Neuroimmunology

W194. Modulation of Chronic EAE and by Oral Administration of miR-30d

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Abstract Text: Background: Fecal microRNAs are an emerging therapeutic area and may act either by direct modulation of the immune system by modulating the microbiome. We found that fecal microRNAs shape the microbiome,1 that miR-30d is elevated in multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE),2 and that oral gavage of miR-30d ameliorates acute EAE, increases Tregs, and expanding Akkermansia muciniphila.2 In this study, we investigated the effects of miR-30d in the chronic animal models of MS and immunologic changes in the CNS. Materials and methods: miR-30d was tested in the acute C57BL6 MOG EAE model and the progressive PLP/SJL adoptive transfer model, which develops cortical lesions, leptomeningeal inflammation and other features shared with progressive MS. EAE was induced and mice were orally gavaged with different concentrations of miR-30d or scramble miR after disease onset. PLP polarized splenocytes were cultured with either miR-30d or scramble and MHC II expression was analyzed with FLOW. Results: 0.3625mg of miR-30d significantly reduced EAE severity in both acute and progressive EAE models. This effect was mediated through the downregulation of MHCII expression in microglia, brain-infiltrating monocytes, and neutrophils. Furthermore, when PLP-polarized splenocytes were cultured with PLP and miR-30d or scramble, miR-30d also downregulated MHCII expression in monocytes and neutrophils *in vitro*. This suggests that miR-30d directly modulates MHCII expression in myeloid cells. Discussion: Oral miR-30d treatment ameliorates EAE by downregulating MHC II and affecting microglia and monocytes, important components of progressive MS. GMP MiR-30d is being manufactured for phase 1 testing in healthy volunteers.

Th180. Myelin Has Less Suppressive Impacts on the Intracerebral Hemorrhage-relevant Alarmin S100A9 Than LPS

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Abstract Text: Introduction: During intracerebral hemorrhage (ICH), blood-derived macrophages that are stimulated by S100A9 produce cytokines that contribute to neuronal death. After three days, these macrophages turn reparative and become essential to tissue repair and recovery. This change in phenotype is poorly understood, but myelin has been implicated in suppressing the macrophage response to different inflammatory signals. Cholesterol, which is abundant in myelin, has been linked to the suppression of macrophages through the transcription factor LXR. Methods and Results: Murine bone marrow-derived macrophages were treated with myelin, cholesterol, or the LXR agonist T091317 for 18 hours. LPS and S100A9 were then used for stimulation for 6 hours. RNA sequencing data revealed that myelin suppressed inflammatory responses while also inducing a myelin-specific phenotype in

macrophages. Secretion of TNF, IL-6, CCL2, and IL-10 was then measured. Myelin and cholesterol suppressed cytokine production during stimulation with LPS, but only to a lesser extent during S100A9 stimulation. T091317 could not suppress cytokine production with any stimulation. Dose titration revealed that neither myelin nor cholesterol suppressed the macrophage response to very high concentrations of LPS. Western blot demonstrated that cholesterol reduced phosphorylation of TLR4 signaling molecules during stimulation with LPS but not S100A9. Conclusions: Myelin's inability to suppress TNF production in response to S100A9 may explain why macrophages are neurotoxic in acute time points of ICH. Cholesterol's inability to replicate myelin's effects suggests other components of myelin may suppress macrophage responses during ICH.

Th181. Selective Depletion of the Microbiota by Antibiotics Effects Survival in the SOD-1 Mouse Model of Amyotrophic Lateral Sclerosis

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Abstract Text: While several genetic risk factors have been identified in the etiology of amyotrophic lateral sclerosis (ALS), the majority of cases present with no known underlying cause, suggesting environmental factors play a role. The gut microbiota is an important environmental factor that shapes disease onset and progression in ALS. We previously found that treating SOD-1 ALS mice with broad spectrum antibiotics worsened the disease and increased microglial inflammation, suggesting that the microbiome as a whole plays a protective role. To investigate the effect of specific microbes, we administered low dose, limited-spectrum antibiotics to female and male SOD mice, including vancomycin targeting Gram-positive bacteria and metronidazole targeting Gram-negative anaerobes. We found that vancomycin an improved survival in female mice, with a median survival benefit of 10 days (p=0.02), suggesting that vancomycin-sensitive bacteria worsen SOD disease progression. Detailed analysis of bacterial populations revealed that vancomycin altered the gut microbiota composition compared to metronidazole and water-treated controls. These microbiota shifts were associated with improved survival and neurological function in female SOD1 mice. In contrast, metronidazole showed no significant impact on disease outcomes. Principal Coordinate Analysis (PCoA) further confirmed distinct gut microbiota profiles across treatment groups, highlighting the potential of microbiota-targeted therapies in ALS management. These findings suggest that specific gut microbiota alterations, particularly those induced by vancomycin, may delay disease progression in SOD1 ALS, likely through mechanisms involving neuroinflammation and immune modulation. Future research will focus on identifying key microbial taxa and metabolites to develop novel therapeutic strategies for ALS.

Th183. Systemic Hyperfibrinogenemia and Neutrophilia Link Respiratory Inflammation with Alzheimer's-associated Neuropathology Relevant to SARS-CoV-2 Post-Sequalae

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Abstract Text: Chronic illness from post-acute sequelae SARS-CoV-2 infection (PASC), is a healthcare burden that is ever-evolving, particularly concerning neurological disease (NeuroPASC). Growing evidence links SARS-CoV-2 infection and an increased risk for neurodegenerative disease, especially in at-risk populations. However, to adequately develop therapeutic strategies for this ensuing healthcare burden, insights into key mechanistic regulators of NeuroPASC are needed. Thus, we will present a potential mechanistic map of key regulatory events that link inflammatory activation of the respiratory tract to neuroinflammatory activation via changes in the circulatory profile of inflammatory regulators. Specifically, we will present our working hypothesis of a regulatory interaction between neutrophils and fibrinogen at the blood-brain barrier (BBB) that alters neurovascular health and ultimately dysregulated microglial function, leading to amyloid aggregation relevant to Alzheimer's Disease (AD). We developed this hypothesis from immunohistochemical observations acquired via high-content microscopy of brain

tissue from wild-type and AD (5xFAD) genetic mouse lines given a combinatorial treatment of intranasal delivery *lipopolysaccharide* (LPS) and retro-orbital delivery of quantum dot virus-like nanoparticles for SARS-CoV-2 (COVID-QDs); this combination served to replicate acute lung injury (ALI) and viremia in circulation, respectively, similar to that observed in moderate to severe COVID-19 clinical cases. We complemented these *in vivo* observations with transwell co-culture systems that recapitulate the interactions between microglia and the BBB. Altogether, these results implicate systemic neutrophilia and hyperfibrinogenemia that damage the neurovascular unit, leading to neutrophil ingress and perivascular deposition of both fibrinogen and COVID-QDs that alter the homeostasis of microglia health and amyloid processing.

Th184. Tau Acetylated PHF6 Reactive T Cells Presented by HLA-DRB1*04:04 in Alzheimer's Disease

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Abstract Text: Background: Neurofibrillary tangles (NFTs) are hallmarks of Alzheimer's disease (AD) and formed by aggregation of the microtube-associated protein tau that undergoes many modifications, including acetylation on lysine (K) in tau. A piece of tau consisting of six amino acid residues, paired helical filament (PHF)6 (VQIVYK), plays a predominant role in tau aggregation. Merging evidence supports adaptive immune system plays an important role. However, the pieces of antigens, the types and functions of T cells, and HLA types associated with AD remain largely unknown. Methods: We analyzed HLA associations in ~176,000 individuals with PD or AD versus controls across ancestry groups. We also compared postmortem brain density of NFTs and amyloid plaques in brain, tau and Aβ42 levels in cerebrospinal fluid (CSF) of ~8,000 individuals (controls and AD) and examined association of HLA in ~2,500 patient with pathologically demonstrated Lewy Body Dementia. This was followed by HLA binding, tetramer T cell studies and TCR activation by tau PHF6 in Jurkat cell. Results: We found that HLA-DRB1*04:04 has protective effect in AD. We detected clonally expanded T cells recognizing tau, especially modified PHF6 with acetylation at lysine 311 (PHF6 aK311) when presented by DRB1*04:04, and the phenotypes of these T cells are distinct in AD patients versus cognitively healthy controls. Further, we identified different TCR clones from both AD patients and healthy controls only activated by tau PHF6 aK311 presented by DRB1*04:04. Conclusion: An HLA-DRB1*04-mediated adaptive immune response against tau PHF6 aK311 decreases AD risk, offering the possibility of new therapeutic avenues.

Tu191. A Risk Variant for Multiple Sclerosis Alters Tr1 Response to Vitamin D

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Abstract Text: Multiple sclerosis (MS) is a complex inflammatory disease of the central nervous system resulting from an intricate interplay between genetic predisposition and environmental factors, such as Vitamin D (VD) deficiency. CD46 costimulation of human $CD4^+$ T cells induces a switch from Th1 to type I regulatory cells (Tr1), characterized by increased IL-10 production. This switch is impaired in MS T cells but can be restored by VD, which also strongly promotes expression of CD226 on CD46-activated T cells. The rs763361 polymorphism in the CD226 gene, resulting in a non-synonymous Gly307Ser variant, is associated with increased risk for MS. Herein, we show that this CD226 risk allele disrupts the ability of CD46-activated T cells to operate the IFN/IL-10 switch upon VD exposure. Mechanistically, the risk variant impairs activation of the integrin LFA-1, which sustains homotypic

adhesion associated with IL-10 production. LFA-1 activation is also impaired in MS T cells expressing the CD226 risk allele upon CD46 and VD stimulation. Furthermore, while MRI activities were independent of the genotype in the placebo group in the SOLARIUM trial, the only 2 pwMS with MRI activities after VD supplementation were homozygous for the CD226 risk allele. Although too small to be conclusive, this supports the hypothesis that the CD226 allele may impact response to VD in patients. Overall, our study unveils how, in the context of MS susceptibility, a genetic polymorphism and an environmental factor act in concert to control the differentiation of Tr1 cells.

Tu192. Alzheimer's Disease-derived *Bacteroides Dorei* Contributes to Cognitive Decline in a Strain-specific Manner

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Abstract Text: The gut microbiota undergoes changes with aging and in Alzheimer's disease (AD), potentially contributing to cognitive decline by modulating immunity. We previously found that *Bacteroides fragilis* exacerbates amyloid-beta (A) plaque deposition by impairing microglial A phagocytosis. Given the functional diversity of bacteria, we explored how different Bacteroides species, depending on strain, might influence A uptake by myeloid cells. We isolated *Bacteroides dorei* from AD and healthy control (HC) subjects and tested its effects on A uptake by RAW macrophages. We found that the HC-derived strain of *B. dorei* stimulated more A uptake than the AD-derived strain. To investigate *in vivo* effects, we administered live HC- or AD-derived *B. dorei* to APP/PS1 and wild-type (WT) mice from 4 to 7 months of age. Cognitive deficits were observed in female mice receiving AD-derived *B. dorei,* which impaired spatial memory. This strain also triggered higher levels of pro-inflammatory cytokines TNF-and IFN-, while HC-derived *B. dorei* reduced Grzb and IL-17 levels. Additionally, bulk RNA sequencing of microglia revealed that AD-derived *B. dorei* increased metabolic processing pathways compared to HC-derived B. dorei. These results suggest that *B. dorei* may have strain- and sex-specific effects on cognition in AD, possibly through an inflammatory response impacting microglia. Understanding the immunologic, metabolomic, and bacterial genomic differences between strains will provide novel insights into how the microbiome contributes to AD pathogenesis.

Tu193. Beyond Bacteria: A Novel Look at the Gut Mycobiome's Role in Multiple Sclerosis

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Abstract Text: Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system with unclear etiology, influenced by genetic and environmental factors. Recent studies have highlighted a potential role of the gut microbiome in MS, with a specific focus on the bacterial components. However, the impact of the gut mycobiota- the fungal community, on immune function and disease remains underexplored. Interestingly, our results show distinct fungal community profiles in patients with MS compared to healthy controls and the manipulation of the mycobiota in experimental autoimmune encephalomyelitis (EAE), a mouse model mimicking many aspects of MS, indicated an important role on gut microbiome, immune function and disease activity. Based on these findings, we hypothesize that gut fungi play an important role on microbiome-immune interactions and thereby could impact the pathogenesis of MS.

Tu194. Brain Fog in Infection-associated Chronic Illnesses: Novel Insights from Neurocognitive and Molecular Profiling of the MAESTRO Clinical Study

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Abstract Text: There is a complex interplay between the host's immune system and pathogens, particularly in the context of Infection-Associated Chronic Illnesses (IACI). These conditions, including those following Lyme disease and COVID-19, represent a growing public health concern with limited diagnostic and therapeutic options. Our clinical study, MAESTRO, employs a comprehensive approach to profile these catastrophic responses in individuals with IACI from Lyme or COVID by conducting neurological assessments, including eye movement tracking, neurocognitive function testing, and hypermobility assessments, incorporated with survey data and deep biological sample (including urine, sweat, saliva, blood, vaginal and rectal swabs) profiling for pathogen loads, dysbiosis, genomics, metabolomics and innovative immune trajectory mapping. The most debilitating and least understood symptom people report in IACI is “brain fog.” Objective measurement and data to support neurocognitive dysfunction are currently lacking. By employing commercially available FDA-cleared devices, such as EEG (Electroencephalogram) and P300 ERP (Event Related Potential) tests through WAVi and eye tracking through RightEye, we are capturing objective measurements of brain function. We have found significant differences in multiple neurocognitive measurements that are unique to IACI. These measurements are being compared to the molecular profiling of the biological samples to identify potential biomarkers associated with brain fog. Our preliminary findings suggest that neuroinflammatory features are significantly correlated with neurocognitive measurements. The use of commercially available tools to measure neurocognitive function has the potential to tremendously improve clinical care for individuals with IACI by providing a more objective and quantitative assessment of poorly understood symptoms such as brain fog.

Tu195. Derangements of the Systemic Immunome in Parkinson’s Disease May Reshape the Microbiome: Possible Pathogenic Relevance on the Gut-brain Axis

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Abstract Text: Background The pathogenic relevance of the gut-brain axis in Parkinson’s Disease (PD) remains poorly understood, despite a growing evidence showing anomalies of the immune system, and reduction of certain species in the gut flora. We hypothesize that the systemic immunome in PD is deranged and targets gut bacteria that may influence dopamine production. Approach We employed high-dimensionality CyTOF and scRNA-seq to explore the systemic immunome in PD patients (n=13), age and gender matched with healthy controls (n=14). Based on a meta-analysis of PD microbiome, we focused on a Lachnospiraceae species which is consistently reduced in PD and potentially linked to dopamine production. We obtained its bacterial extract and engineered a

protein encompassing the catalytic site of SadA, a key protein for dopamine synthesis, using both as targets for *in vitro* immune responses. Results The immunome architecture in PD differed significantly from healthy subjects, with network relationships between regulatory and inflammatory subsets indicating an imbalance. An aberrant, species-specific, pro-inflammatory response was detected, marked by SadA-specific cytotoxic T cells ($CD8A^+$ $NKG7^+$), expressing CCL4 and suggesting transit through gut mucosa. Pro-inflammatory monocytes expressing CD93, S100A8, S100A9, and integrin receptor ITGB8, also suggesting their transit through the gut mucosa, were detected after stimulation with bacterial extract. Conclusion Our data opens a novel perspective on the gut-brain axis in PD, highlighting immune aberrations, that drive the ablation of bacterial species, probably useful in reducing the dependency of endogenous dopamine production, which is inherently impaired in PD.

Tu196. Epigenetic Regulation of Microglia and Infiltrating Myeloid Cells in the Central Nervous System of Mice with Experimental Autoimmune Encephalomyelitis

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Abstract Text: Introduction: Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of the central nervous system (CNS), causing damage to myelin, oligodendrocytes, and axons. Early stages involve lymphocyte and macrophage infiltration into the CNS, persisting at reduced levels later. Microglia, the CNS's tissue-resident macrophages, are activated early and become increasingly involved as the disease progresses. Their role in MS is complex, balancing pro-inflammatory activity with benefits like phagocytosing neurotoxins and myelin debris, and supporting oligodendrocyte progenitor cells. This study investigates longitudinal changes in microglia and infiltrating myeloid cells in the CNS of mice with experimental autoimmune encephalomyelitis (EAE), an archetypical MS animal model. Methods: We collected microglia and myeloid cells from the CNS at four stages (baseline, acute, remission, chronic) in SJL/JRj mice with EAE. DNA and RNA were isolated for methylation analysis and bulk RNAseq, respectively. Results: Analysis of DNA samples using SeSAMe identified 9504 (delta-beta ≥ 0.1; FDR ≤ 0.05) and 11524 (delta-beta ≥ 0.2; FDR ≤ 0.05) differentially methylated positions in microglia and myeloid cells, respectively. A heatmap visualized and clustered the data. Gene ontology analysis was conducted with GREAT, and transcription factor binding motif enrichment was analysed using HOMER. Bulk RNAseq is currently being analysed with results expected soon. Conclusions: Significant changes were observed during the acute phase and progression to chronic phase, affecting genes involved in microglia and myeloid cell activation during clinical relapse in the EAE model. *In vitro* validations of our results are presently being set up to continue our research.

Tu197. Gamma-delta T Cells Exacerbate Neuroinflammation in a Mouse Model of Alzheimer's Disease by Modulating Microglial Cells

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Abstract Text: Neuroinflammation is a central driver of Alzheimer's disease (AD), with microglia transitioning from a homeostatic (M0) to a neurodegenerative (MGnD) phenotype, further amplifying inflammation. MGnD microglia recruit and activate peripheral immune cells, including CD8 T cells, contributing to neuroinflammation and neuronal death. Gamma-delta (γδ) T cells, a unique subset of T lymphocytes, sustain neuroinflammation by secreting pro-inflammatory cytokines such as IFN-γ and IL-17. In a traumatic brain injury (TBI) model, we found that γδ T cell-deficient ($γδ^{-/-}$) mice exhibited fewer amyloid plaques and improved cognitive functions. Vγ4 γδ T cells, producing IFN-γ and IL-17, exacerbated inflammation, while Vγ1 γδ T cells, producing TGF-β, mitigated it. These findings led to

the hypothesis that γδ T cells drive microglial activation toward the MGnD phenotype, recruiting and activating peripheral immune cells, thereby perpetuating neuroinflammation and neurodegeneration. Accordingly, scRNA-seq analysis of public datasets showed enriched IFN-γ and IL-17 signatures in γδ T cells from PBMCs and brains of AD patients. In the APP/PS1jucker AD model, both Vγ1 and Vγ4 γδ T cells infiltrated the brain, coinciding with an MGnD microglial phenotype and increased CD8 T cells. APP/PS1:$γδ^{-/-}$ mice exhibited improved cognition, reduced amyloid plaques, a shift in microglial phenotypes from MGnD to M0, and fewer CD8 T cells. These findings suggest that γδ T cells, particularly Vγ1 and Vγ4, play a detrimental role in AD by driving neuroinflammation through interactions with microglia and CD8 T cells. This study may build on the growing number of clinical studies exploring γδ T cells as therapeutical tools.

W200. High-Throughput Identification and Characterization of Patient-Derived Monoclonal Autoantibodies in Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease (MOGAD)

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Abstract Text: Myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease (MOGAD) is a CNS demyelinating autoimmune disorder characterized by autoantibodies targeting MOG. However, the mechanisms by which these autoantibodies mediate disease pathology remain unclear, limiting treatment options for patients. To address this knowledge gap, we aim to understand the immunopathology of autoantibody-mediated MOGAD by producing and studying patient-derived monoclonal antibodies (mAbs), and characterizing their somatic mutational load, clonal expansion, isotype, and binding strength—indicators of antigen-driven maturation and potential Fc-mediated pathology. However, understanding the heterogeneity of autoantibody responses across MOGAD patients necessitates generating a large library of mAbs, and consequently a high-throughput approach. Accordingly, we implemented an optofluidic-based antigen-specific method on the Beacon platform to identify and isolate patient-derived MOG-specific B cells, from which B cell receptor (BCR) sequences were used to generate mAbs. We identified B cells from three patients producing MOG-binding IgG antibodies and found 16 unique pairs of BCR heavy and light chain sequences. Using this data, we generated mAbs and tested their binding specificity using a live MOG-expressing cell-based assay (CBA) and MOG-based ELISA. One mAb, with high mutational frequency, tested positive on the ELISA. Additional mAbs are being tested by CBAs for binding and Fc-mediated effector functions. Herein, we present a high-throughput strategy that has the potential to expedite antibody discovery workflows for rare pathogenic cell types, advancing our mechanistic understanding of MOGAD and enabling patient-specific therapeutic strategies and improved clinical outcomes.

W201. Imbalance of γδ T and MAIT Cells and Defective αβ TCR Clonality in Poorly-Controlled Epilepsy

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Abstract Text: One-third of epilepsy cases are refractory to anti-seizure medications, highlighting the urgent need for novel therapeutic strategies. Mounting evidence has linked blood-brain barrier (BBB) damage and immune cell regulation to epilepsy, but the immune mechanisms underlying treatment resistance remain elusive. We conducted the first immunomics in the blood and brain of poorly-controlled (PC) and well-controlled (WC) epilepsy using single-cell RNA and T cell receptor (TCR) sequencing of peripheral (n=106) and brain-infiltrating (n=10) immune cells, coupled with plasma proteomics and metabolomics analyses. Our findings revealed striking alterations in T cell subsets: Gamma-delta (γδ) T cells were enriched in PC patients, while mucosal-associated invariant T (MAIT) cells

were reduced in PC patients, suggesting opposing roles of these innate-like T cell populations in epilepsy. PC patients exhibited reduced alpha beta (αβ) T cell clonality, correlating with shorter seizure-free intervals and heightened depression risk. Intriguingly, we identified five TCR clusters shared among epilepsy patients all targeting a cytomegalovirus antigen, implicating viral antigens in driving immune dysfunction. Plasma proteomics implicated impaired coagulation and wound healing pathways in PC patients, indicative of persistent BBB disruption. Brain tissue analysis unveiled reduced T cell infiltration in PC patients compared to post-mortem brain tissue from non-epileptic controls. Remarkably, activated and clonally expanded T cells emerge as potential protectors against epilepsy. These discoveries not only redefine epilepsy as an immune-driven disorder but also unveil antigen-specific T cells as a transformative avenue for refractory cases.

W202. Investigating Microbiota-immune Interactions in the 3KL Model of Parkinson's Disease

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Abstract Text: The gut microbiome and inflammation are implicated in Parkinson's disease (PD), yet the specific microbial players and immunologic mechanisms remain unclear. To explore how gut microbes influence PD progression, we used individual antibiotics to deplete specific bacterial populations in the 3KL mouse model of PD. Mice were treated with metronidazole, neomycin, or penicillin from 7-12 months of age, followed by motor function testing. Longitudinal stool samples were analyzed via 16S rRNA sequencing, and microglial responses were characterized using RNA sequencing. We found that metronidazole slowed motor dysfunction progression in male 3KL mice, measured by hindlimb clasping. 16S sequencing revealed that metronidazole selectively depleted *Alistipes muris,* but not *Alistipes* in mice treated with neomycin or penicillin. KEGG pathway analysis of microglial RNA sequencing indicated that metronidazole altered disease-relevant processes such as PD and mitophagy, and modulated immune processes including Th17 cell differentiation and IL-17 signaling. These findings suggest that metronidazole slowed motor dysfunction by reducing *Alistipes* and dampening peripheral inflammatory responses, along with altering microglial transcriptional profiles associated with PD and inflammation. Further experiments with *Alistipes muris* revealed that its administration worsened the motor phenotype, increased pro-inflammatory cytokine production, and enhanced $CD8^+$ T cell IFN-γ and γδ T cell IL-17 secretion. These results suggest that *Alistipes,* abundant in PD, exacerbates disease through its effects on microglia and peripheral immunity, particularly via IL-17 signaling, offering potential targets for therapeutic intervention.

W203. Less Precise Medicine - An Argument for Broad-kinase Inhibition in a Microglial Model of Parkinson's Disease

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Abstract Text: As a kinase hub in the primary immune effector cell of the brain, leucine-rich repeat kinase 2 (LRRK2) is an attractive target for precision medicine; the selective inhibition of this malefactor should attenuate the major pathology of Parkinson's Disease (PD). However, kinases are important regulatory nodes in signaling cascades, and complex co-regulatory networks exist between them to maintain cellular health. We posit that a less precise approach via broad, yet still targeted, inhibition of multiple kinases within a co-regulatory kinome can synergistically produce therapeutic activity at lower doses. For example, both LRRK2 and mixed-lineage kinase 3 (MLK3) are commonly implicated across various neurodegenerative diseases. Thus, using bulk RNAseq in microglia-like BV-2 cells, we screened for differential regulation of key biological processes in response to LRRK2 inhibition (LRRK2-IN-1), MLK3 inhibition (MLK-IN-1), or dual inhibition (URMC-099). All three inhibitors attenuated inflammatory activation from pre-formed fibrils (PFFs) of α-synuclein (aSyn), the primary component of Lewy Body

aggregates reported in PD. Of note, only URMC-099 returned cells to basal conditions, while inhibition of either LRRK2 or MLK3 excessively downregulated multiple cellular processes involved in homeostatic microglial function. We corroborate our transcriptomic findings with dose-response resazurin viability assays and immunocytochemical analysis. In aggregate, our data suggest that selective inhibition of only LRRK2 in microglia dysregulates transcriptional regulatory machinery and, paradoxically, excessively disrupts autophagic processing necessary for chronic attenuation of stress induced by aSyn PFFs. Targeted, broad kinase inhibition presents an alternative therapeutic mechanism for PD, with reduced dysregulation of the brain's primary immune effector cell.

W204. MiRNA-21 Control of Type I Interferon Signaling in CNS Autoimmunity

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Abstract Text: IFN-beta, a type I interferon, has been used as a first-line therapy for patients with multiple sclerosis (MS) for thirty years; however, the cellular and molecular basis of its therapeutic efficacy remains largely unknown. Here, we report that IFN-beta and type I IFN signaling limits central nervous system autoimmunity by inhibiting microRNA-21 (miR-21)-mediated pathogenic Th17 development. Briefly, IFN-beta limits miR-21 expression within $CD4^+$ T cells, including miR-21-mediated pathogenic Th17 development, by downregulating miR-21-inducing cytokines within myeloid cells. We show miR-21 itself promotes pathogenic Th17 differentiation by inhibiting a key transcription factor, Foxo1. Accordingly, miR-21 loss abrogates pathogenic Th17 differentiation and confers resistance to experimental autoimmune encephalomyelitis, a mouse model of MS. In patient samples, we found that compared to responders of IFN-beta treatment, non-responders express elevated miR-21-inducing cytokines within myeloid cells, alongside increased miR-21 and pathogenic Th17 cytokines within $CD4^+$ T cells. Finally, direct miR-21 inhibition reduces pathogenic Th17 differentiation in non-responder $CD4^+$ T cells. These findings provide insights into the mechanisms underlying IFN-beta therapy in MS patients, suggesting that miR-21 may be a potential biomarker to predict therapeutic response, and raises the possibility that miR-21 inhibition may be of potential therapeutic value specifically for the IFN-beta non-responder cohort.

W124. Sex Bias in Tr1 Differentiation and IL-10 Production: Role of PI3K Signaling in Multiple Sclerosis

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Abstract Text: Multiple sclerosis (MS) is a chronic inflammatory disease with a higher prevalence in females. T cells are key in MS, with CD46 converting Th1 cells into type 1 regulatory T cells (Tr1). This pathway is impaired in MS, reducing IL-10 production. The relationship between sex and Tr1 differentiation remains unexplored. Our data show a sex bias in Tr1 differentiation upon CD46 activation in healthy donors. Female CD46-induced Tr1 cells produced lower levels of IL-10 and IFN-g, with reduced c-MAF expression, compared to males. This sex bias was not restricted to the CD46 pathway, as a similar sex bias was observed in IL-27-induced Tr1 cells. As it was previously shown that PI3K pathway inhibition in IL-27-induced-Tr1 cells inhibited IL-10 secretion in mice, we examined the role

of this pathway in human Tr1 cells. Surprisingly, we observed an antagonistic role for PI3K depending on the Tr1 induction pathways, as well as a sex bias in the response to inhibition. While PI3K inhibition decreased IL-10 production by IL-27-induced Tr1 cells, it increased IL-10 production in CD46-mediated Tr1 cells, with a stronger effect in females. Differences in PI3K pathway could explain the sex-based difference in IL-10 production. Interestingly, the sex bias in CD46-induced Tr1 cells was not observed in people with MS. However, PI3K inhibition restored IL-10 production in both sexes, suggesting potential new therapeutic strategies. Overall, our data demonstrate that sex regulates IL-10 production by CD46-induced Tr1 cells through the PI3K pathway, potentially contributing to female susceptibility to MS and autoimmune diseases.

Reproductive Immunology

Th185. Chronic Exposure to Glyphosate-based Herbicides Compromises Semen Quality, Which in Turn Disrupts the Immunoregulation of the Female Genital Tract and Affects Fetal Development

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Abstract Text: The widespread use of glyphosate-based herbicides (GBH) has raised significant concerns about their potential impact on reproductive health. However, most available data remain controversial, leaving a significant gap in understanding GBH's specific effects on male fertility in humans. This study aimed to evaluate sperm quality and semen inflammation in men chronically exposed to GBH. Additionally, an animal model was used to investigate GBH's effects on male fertility as well as embryonic and placental development. Sperm quality in human patients was analyzed following WHO guidelines. Semen levels of cytokines and leukocyte subsets were quantitated by flow cytometry. In parallel, male C57BL/6 mice were orally treated with GBH or water for 35 days. Males were then mated with BALB/c females. Sperm quality, different fertility parameters, and placental development were analyzed. Moreover, uterine immune cell infiltration was evaluated at the peri-implantation window. Men exposed to GBH exhibited reduced sperm concentration, motility, morphology and significant semen inflammation characterized by increased levels of leukocytes and cytokines. Similarly, GBH-exposed mice showed reduced sperm quality. Interestingly, females mated with these males showed significant uterine inflammation during the peri-implantation window, evidenced by increased infiltration of macrophages and CD4 and CD8 T lymphocytes. Strikingly, pups sired by GBH-exposed males showed altered embryonic and placental development, with reduced fetal and placental sizes. These findings indicate that chronic exposure to GBH adversely affects semen quality, which in turn hinders the sperm's ability to physiologically induce immunoregulation in the female genital tract. This impairment also negatively impacts placental and fetal development.

Th186. Shared and Distinct Placental Immune Dynamics Across Pregnancy Complications

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Abstract Text: The placenta is the heart of the maternal-fetal interface and is composed of cells that allow flow of oxygen and nutrients between mother and fetus while maintaining immune tolerance. High-dimensional techniques have been applied to understand placental cellular dynamics in individual pregnancy complications, but a comprehensive map of the maternal-fetal interface across all immune-mediated pregnancy complications has never been constructed. Understanding the complex cellular dynamics that govern tolerance (or lack thereof) and other critical biology at the maternal-fetal interface in healthy and complicated pregnancy is key to developing effective diagnostic and therapeutic strategies. To this end we collected 69 placenta samples from patients with a healthy pregnancy (labored n=9, unlabored n=13) as well as patients with pregnancy complications including spontaneous preterm birth (n=10), fetal growth restriction (n=9), pre-term preeclampsia (n=10), term preeclampsia (n=8) and type 1 diabetes (n=10). Male and female fetal sex were balanced across groups, to permit evaluation of sex differences on placental cellular programs. We profiled all samples by multi-modal single cell sequencing including gene expression, surface protein (CITE-seq) and paired single cell T cell receptor sequencing. We profiled over one million placental cells, creating the largest placenta single cell atlas to date. We identified various cellular lineages by unsupervised clustering. We characterized cellular programs, cell abundance changes and cell-cell communications, identifying the features that are shared and different across different pregnancy complications. This unprecedented biological and computational resource is urgently needed to guide evidence-based diagnostics and therapeutics that can improve maternal and fetal health.

Tu198. Disruption of Placental Macrophage Function and Vascular Development by First-line Antiretroviral Drugs: Rescue Potential of Exogenous FXIIIA1

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Abstract Text: Background: We previously reported that antiretroviral treatment (ART) in pregnant individuals living with HIV is associated with a 2-fold increased risk of maternal vascular malperfusion. We also found lower levels of coagulation factor XIIIA1 (FXIIIA1) in placental Hofbauer Cells (HBCs), which correlated with pre-term birth, particularly when ART was initiated pre-conception. We hypothesize that ART disrupts HBC function through FXIIIA1, impairing placental vascularization. Methods: We employed a HUVEC angiogenesis assay to investigate FXIIIA1 and its inhibitor ZED1301. Placenta-like macrophages were generated by stimulating primary human monocytes with M-CSF and IL-10. We tested ARV cytotoxicity, determining IC50 and physiological concentrations for co-culture experiments with HUVECs and macrophages. Results: FXIIIA1 (5 µg/ml) enhanced branching and tubular formation in the HUVEC model (p=0.04). Inhibition of FXIIIA1 with ZED1301 reduced branch thickness in a dose-dependent manner. The important role placental macrophages in this process was confirmed when placenta-like macrophages promoted vascular growth without conventional endothelial growth factors (p=0.0016). However, when pre-treated for 24h with dolutegravir or a Tenofovir + 3TC +Efavirenz combination, these macrophages no longer supported angiogenesis, showing reduced tube formation and network connectivity (p=0.0013). A similar effect was observed when ARVs were added directly to the HUVEC model. Exogenous FXIIIA1 rescued angiogenesis impaired by ARVs. Conclusion: These findings suggest that pre-conception ART may impair placental vascularization by disrupting HBC function through FXIIIA1. This provides a potential mechanism by which ART may contribute to adverse birth outcomes and highlights the need for further investigation into adjunctive therapies to counteract these effects.

Tu199. Semen Inflammation to Fertility Challenges: Unraveling the Immune Cross-talk Between Male and Female Genital Tracts and Its Impact on Offspring

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Abstract Text: Male infertility accounts for up to 50% of infertility cases and urogenital inflammation has been proposed as a cause. Chronic prostatitis patients show semen inflammation and reduced sperm quality. Given that the seminal fluid is crucial not only for fertilization but also for inducing immunoregulation in the female genital tract (FGT) to support embryo implantation and pregnancy development, chronic prostatitis could have further effects on reproduction. Herein, we aimed to investigate the impact of Experimental Autoimmune Prostatitis (EAP) on male fertility, immunomodulation in the FGT, and offspring development. EAP was induced in C57BL/6 male mice. The prostate-specific immune response, prostate histopathology, and sperm quality were evaluated. Mating experiments with female BALB/c mice were performed to assess fertility parameters, uterine immune cell infiltration and cytokine/chemokine expression, and offspring development. EAP males exhibited chronic prostate inflammation, semen inflammation and oxidative stress, and reduced sperm quality. Females mated with EAP males showed significantly increased uterine leukocyte infiltration, upregulated expression of IL-1β and IL-17A, along with reduced IL-10, and downregulated expression of embryotopic factors (LIF, IL-6 and IGFBP-1) whereas increased expression of the pro-apoptotic factor TRAIL. Additionally, females mated with EAP males showed significantly lower fertility indexes, and higher rates of embryo loss. Strikingly, offspring sired by EAP males exhibited reduced growth and decreased sperm quality when adults. Thus, chronic prostatitis causes semen inflammation and disrupts immunoregulation in the FGT, compromising fertility and negatively impacting offspring development. These findings highlight the importance to properly assess male urogenital inflammation to safeguard fertility and offspring health.

W206. Expression Features of Foxp3 in Eutopic Endometrium of Women with Infertility Associated with Endometriosis

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Abstract Text: Background: An important cause of female infertility is endometriosis—a disease characterized by the ectopic proliferation of the endometrium. Therefore, endometriosis resembles "autologous transplantation" with "immunological tolerance" to ectopic endometrium, which indicates a violation of immunological regulation. There is a lot of evidence about the important role of immunological regulation for the onset of a successful pregnancy, but the causes of infertility in endometriosis are not entirely clear, especially regarding the role of regulatory endometrial cells, which determined the goal of our study—to evaluate the expression of the transcription factor gene regulating the differentiation of Treg cells—Foxp3 in the tissue of the eutopic endometrium in women with infertility associated with endometriosis. Methods: Samples of endometrial tissue were collected from 40 women with infertility associated with endometriosis (main group) and 20 women with tubal infertility (control group). The expression of the Foxp3 gene was determined by real-time PCR. Results: The expression of the Foxp3 gene in the endometrial tissue of women with infertility and endometriosis was significantly reduced compared to the control group ($p<0.001$). At the same time, there was no statistically significant difference in Foxp3 gene expression between groups of patients with mild, moderate, and severe stages of endometriosis ($p>0.05$). Conclusion: The reduction of Foxp3 gene expression in the endometrial tissue of women with infertility associated with endometriosis may be one of the factors of infertility, although the role in the progression of endometriosis remains unclear. Keywords: infertility, endometriosis, immunoregulation, Foxp3.

W207. Lyme Disease Increases Risk for Multiple Gynecological Conditions with Age

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Abstract Text: Lyme disease (LD) is an illness caused by the spirochete Borrelia burgdorferi (Bb). Bb disseminates through organs, including skin, joints, spinal cord, bladder, and heart, leading to Lyme arthritis, neuroborreliosis, and Lyme carditis. While previous studies have investigated the impact of LD on pregnancy in both mice and humans and have found the presence of Bb in the uterus of mice, we studied the impact of LD on the non-pregnant female reproductive tract (FRT). We use a LD mouse model and find an ongoing and severe infection of the FRT, which persists up to 15-months post-inoculation. This infection results in uterine glandular cysts, endometrial hyperplasia, and interspersed endometrial stromal cells as well as vaginal epithelial thickening, polymorphonuclear and mononuclear cell epithelial infiltration, and canal lumen epithelial desquamation. Even in C57BL/6 mice, long thought to be asymptomatic for LD, we detect FRTs infected with Bb after 8-weeks of infection, and ongoing infection throughout the next 12-months in n=5/6 individuals. Additionally, we find that age has an impact on the extent of gynepathology such that aged mice (1-year old) that are reproductively senescent have more gynepathology with infection compared to young mice (15-weeks old) when infected for the same length of time. Using large-scale electronic healthcare record data, we report that LD additionally results in increased infection-associated risk of menorrhagia, miscarriage, uterine fibroids), and endometriosis. This work suggests that further study of the FRT and the effects of Bb infection therein will help clarify and expand our knowledge of LD outcomes.

Stem Cell and Organ Transplantation

Th194. Combinatorial Genetic Engineering Strategy for Antigen-specific Protection of Stem Cell-derived Beta Cells by Chimeric Antigen Receptor Regulatory T Cells

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Abstract Text: Regenerative medicine harnesses human pluripotent stem cell (hPSC)-derived cells and tissues to treat many diseases, including autoimmune disorders. Yet, immune protection of grafts remains a critical challenge for widespread implementation. Chimeric antigen receptor (CAR) technology can confer new antigen specificities to effector T cells and, more recently, regulatory T cells (Tregs), potent immunosuppressive cells. However, a key challenge in CAR design is identifying a target molecule uniquely expressed by the cells of interest to maximize efficacy and safety. We developed a combinatorial genetic engineering approach to confer CAR-Treg-mediated localized immune protection to stem cell-derived cells using a humanized type 1 diabetes preclinical mouse model.

hPSCs were modified to express a truncated epidermal growth factor receptor (EGFRt), a biologically inert local target for CAR-Treg homing and activation. In parallel, we generated EGFR CAR Tregs were generated. EGFR CAR Tregs were activated by EGFRt-expressing hPSC-derived pancreatic beta-like cells (sBC) and suppressed innate and adaptive effector immune responses *in vitro*. Strikingly, EGFR CAR Tregs, but not polyclonal Tregs, protected transplanted EGFRt-expressing insulin-producing sBCs from CAR-T cell-mediated destruction in NSG mice in the absence of any additional gene edits in the sBCs. Towards translation into the clinic, we have developed and validated a new engineered inert cell surface ligand and a CAR specific for it. Ongoing experiments aim to evaluate this ligand-CAR pair in a humanized mouse model of type 1 diabetes, dissect the mechanisms of CAR Treg-mediated graft protection, and explore synergies with immunoregulatory gene edits in hPSCs.

Tu200. Achieving Allograft Protection Through Co-implantation with Donor Antigen-primed Lymph Node Stromal Cells

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Abstract Text: Objective/Background: Pancreatic islet transplantation for type 1 diabetes requires lifelong immunosuppression, reducing its applicability. We aim to reduce the need for immunosuppression by co-transplanting lymph node fibroblastic reticular cells (FRCs). FRCs are unconventional antigen-presenting cells that contribute to peripheral tolerance and could be used in transplantation to regulate alloreactive T cells. We hypothesize that autologous FRCs co-transplanted with allogeneic donor islets can uptake and present alloantigen to induce alloreactive T cell anergy and/or regulation. Methods: C57BL/6 FRCs were pre-conditioned with 10ng/mL IFNγ for 72h. FRCs were incubated with CellTrace-labeled NIT1 (fully mismatched to B6) cell lysate, 50μM chicken ovalbumin257-264 (SIINFEKL) peptide, or 1mg/mL ovalbumin (OVA) overnight. For kinetics, FRCs were cultured in regular media after overnight treatment. Flow cytometry was used to detect labeled lysate uptake and antigen presentation with anti-SIINFEKL-H2Kb. SIINFEKL or OVA antigen-primed FRCs were co-cultured with SIINFEKL-reactive cells and activation was measured with β-galactosidase reporter quantification or flow cytometry. Results: FRC uptake of fluorescent NIT1 lysate was dose-dependent, and 96.3±2.6% of FRCs took up lysate at high dose. Confocal microscopy demonstrated lysate localization within lysosomes. Donor antigen was cleared from FRCs by 72h. IFNγ potentiates SIINFEKL presentation and 99.9±0.1% of IFNγ-treated FRCs presented SIINFEKL. SIIFNEKL-reactive cells were activated by SIINFEKL- and OVA-primed FRCs. Conclusion: *In vitro*, FRCs can acquire exogenous peptide and protein antigen, process and present them in MHC-I, and activate $CD8^+$ cells. These results predict that FRCs can uptake allogeneic donor cell antigens and present them to alloreactive recipient T cells in *in vivo* co-transplantation.

Tu201. Human Microbiota-reactive dp8a Regulatory T Cells Protect Against Transplantation-related Disorders

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Abstract Text: Alteration of intestinal microbiota, called dysbiosis, is associated with numerous pathologies, including complications following allogeneic transplantations like acute graft-versus-host disease (aGvHD) and

kidney transplant rejection. These conditions, have been associated with a low abundance of the commensal Faecalibacterium duncaniae (F. duncaniae) in patients' stools. Interestingly, our team has identified a Treg subset, called DP8a, reactive to F. duncaniae and whose suppressive function rely on the CD39- and CD73-dependent purinergic pathway, which had also been implicated in these two disorders. Using two cohorts (507 and 230) kidney-transplanted patients (DIVAT and ORLY-Est, respectively), we observed that $CD73^+$ DP8a Tregs were significantly increased post-transplantation in patients who will not reject their graft, as compared to patients who will reject it later on, making those cells both a potential predictive biomarker and a therapeutic tool. This increase was not observed pre-transplantation, indicating that DP8a Treg activation occurred upon transplantation. In another transplantation context, using a cohort of 62 patients treated by allogeneic stem cell transplantation, we observed specific alterations of the DP8a Treg subset in patients who developed aGvHD. Remarkably, not only donor-derived $CD73^+$ DP8a Tregs were decreased, but also displayed lower host-reactivities in aGvHD patients, as compared to aGvHD-free patients. Cloning alloreactive DP8a T cells demonstrated that they could cross-react with F. duncaniae antigens and harbored potent regulatory functions. Altogether, these results strongly support that the DP8a Treg subset could play a key role in tolerance to allogeneic transplantation, possibly through their cross-reaction(s) with alloantigens, hence providing anti-inflammatory protection against these transplantation disorders.

W208. Immunosuppressive Potential of hUC-MSCs: Modulating Macrophage-Driven Inflammation Through Direct and Paracrine Mechanisms

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Abstract Text: Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) are emerging as potent immunomodulators with significant therapeutic potential in inflammatory and immune-mediated diseases. Macrophages, particularly the pro-inflammatory M1 phenotype, play a pivotal role in mediating pathological inflammation by producing nitric oxide (NO) and inflammatory cytokines. This study investigates the effects of human umbilical cord-derived MSCs (hUC-MSCs) on inflammation and nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW264.7 macrophages. The Griess assay demonstrated that LPS stimulation significantly increased NO production, whereas co-culture with hUC-MSCs led to a time- and dose-dependent reduction in NO levels. The highest MSC ratio (1:2) resulted in a significant decrease in NO, with conditioned media (CM) showing a greater NO-suppressive effect than direct co-culture. Cytokine analysis revealed the dynamic immunomodulatory effects of hUC-MSCs. While MSC co-culture initially suppressed TNF levels at 12 hours, this effect was not sustained, with TNF increasing at later time points. IL-6 levels were significantly elevated at 12 hours but showed a delayed suppressive effect at 24 hours. MCP-1 was consistently reduced across all co-culture conditions, indicating prolonged suppression. IL-10 secretion was significantly increased at 12 hours, with sustained elevation at 24 and 48 hours, particularly in direct co-culture groups. However, IL-12p70 and IFN-γ levels remained largely unaffected. These findings highlight the potential of hUC-MSCs to modulate inflammation through NO inhibition and cytokine regulation, suggesting their therapeutic utility in inflammatory lung conditions.

W209. Matching of Major Histocompatibility Complex Class I Is Necessary but to Sufficient for Tolerance Induction to Vascularized Composite Allografts Following Donor Hematopoietic Stem Cell Transplantation

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Abstract Text: Hematopoietic stem cell transplantation (HSCT) can facilitate tolerance of concomitantly transplanted tissue. Previous research in a preclinical porcine model has evaluated tolerance development after combined HSCT/vascularized composite allografts (VCA) across various major histocompatibility complex (MHC) class I and class II barriers. In the present study, we used the same preclinical model to further investigate the relative importance of class I versus class II matching for induction of tolerance to tissue-specific skin antigens in a situation where the recipients are made tolerant to donor MHC through HSCT. Recipient pigs received CD3 immunotoxin and total body irradiation followed by HSCT and VCA transplantation from the same donor. Cyclosporine immunosuppression was discontinued after 45 days. Our results indicate that combined HSCT/VCA transplantation between fully MHC class I matched pairs generates durable multi-lineage mixed chimerism in peripheral blood and tolerance of both donor MHC and tissue-specific antigens, regardless of degree of class II matching. When MHC class I is fully mismatched, however, the degree of durable mixed chimerism in peripheral blood, bone marrow engraftment, and rejection correlates with the number of mismatched class II alleles. When a single class I allele is matched, combined HSCT/VCA generates durable multilineage mixed chimerism, but consistent tolerance of both donor MHC and tissue-specific antigens occurs only when just a single class II allele is also matched. Our results suggest that, in the absence of full class I matching, the development of tolerance is influenced by the degree of class II matching in a manner consistent with linked suppression.

W210. Mechanistic Studies in a New Tolerance Trial of Combined Kidney and Bone Marrow Transplantation

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Abstract Text: Tolerance was achieved in a small cohort receiving combined kidney and bone marrow transplantation (CKBMT) with transient mixed chimerism. Cytokine release syndrome causing renal dysfunction led to modification of the regimen by increasing anti-CD2 antibody and adding a short course of MMF. In the previous trial, donor-reactive T cell clones (DRTCCs) were identified via high throughput TCR sequencing and tracked post-transplant. Loss of DRTCCs and expansion of donor-specific Tregs correlated with tolerance. In the current protocol, high-throughput CDR3-TCRβ sequencing on DNA extracted from FACS-sorted CFSE-low $CD4^+$ and $CD8^+$T cells isolated from pre-transplant mixed lymphocyte reactions (MLRs) and unstimulated PBMCs identified DRTCCs. These were tracked in unstimulated CD4 and CD8 populations at months 4, 12, 24. The number of circulating CD4 and/or CD8 DRTCCs decreased post-transplant in all patients. When normalized to their pre-transplant total frequencies, all patients except Pt2 who had a higher CD8 relative ratio, showed a relative reduction (< 1) or a relative ratio around 1 in circulating CD4 and/or CD8 DRTCCs by 4 months post-transplant (Pt1 CD4: 0.03, CD8: 0.09; Pt2 CD4: 0.8, CD8: 2.6; Pt3 CD4: 1.2, CD8: 0.5; Pt5 CD4: 1.4, CD8: 0.5). Transient multilineage donor chimerism was observed in all patients, peaking at 12-70% and decreased to < 1% by 30 days in three patients. Circulating Tregs were enriched (up to 79.5%), then gradually declined to baseline by 7.5-12 months. Our data indicates an early loss of DRTCCs in CKBMT recipients. Ongoing studies of tolerance and donor-specific Treg sequences will identify mechanistic correlations of outcomes.

W211. Rejection of Synthetic Human Skin Can Be Triggered by Donor Endothelial Cell Presentation of Alloantigen

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Abstract Text: In contrast to vascularized skin, avascular synthetic skin constructs containing human fibroblasts and keratinocytes allogeneic to the recipient are not rejected clinically or by adoptively transferred allogeneic human

PBMCs in immunodeficient mice. The presence of graft endothelial cell (EC)-lined microvessels may function to activate T cells or simply provide a conduit into the tissue. We generated 3D-printed human skin using fibroblasts, pericytes, keratinocytes and ECs from a single donor using ECs that were either unmodified or deleted of class I and II HLA antigens and implanted them on adult MISTRG6 mouse that had received adult hematopoietic stem cells (HSCs) as neonates, Mice were inoculated 2 weeks following skin grafting with PBMCs from the same donor as the HSCs. HSC-engrafted mice developed circulating myeloid and lymphoid cells; circulating human T cells dramatically increased after PBMC inoculation. Mice engrafted with HSCs-only did not reject grafts. Grafts with HLA^+ ECs became infiltrated by human macrophages, $CD4^+$ and $CD8^+$ T cells progressively over three weeks following PBMC inoculation. $CD8^+$ cells expressed granzyme B and grafts displayed both EC activation (E-selectin and VCAM-1 expression) and vascular injury indicative of rejection. In contrast, grafts formed using HLA- ECs were not rejected and lymphocyte infiltration was minimal despite having comparable vascularization. We conclude HLA^+ presentation by ECs is necessary and sufficient to trigger human allograft rejection in the absence of passenger leukocytes. Our model can be used to study the roles of specific human graft cells and molecules in rejection.

Systems Immunology

Th121. Mapping T Cell Antitumor Responses in Whole Blood to Solid Tumor Tissue Biopsies of Cancer Patients Using Mass Cytometry

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Abstract Text: Immunotherapy has revolutionized cancer treatment, yet it exhibits limited efficacy in solid tumors due to their complex immune landscape involving an immunosuppressive tumor microenvironment (TME). To develop safe and effective immunotherapies, it is crucial to achieve a comprehensive understanding of localized and systemic immune responses. Here, we took an integrative approach of mapping localized and systemic T cell responses with CyTOF™ and Imaging Mass Cytometry™ (IMC™) technologies in matched PBMC, tumor-derived cells and FFPE tumor tissues from cancer patients. Samples were barcoded, stained with a 40-plus-marker CyTOF panel (including lineage markers, immune checkpoint markers, cytokines and transcription factors), frozen and acquired later using a CyTOF XT system. FFPE tumor tissues were stained with a 40-plus-marker immuno-oncology IMC panel to phenotype immune, stromal and tumor cells, and assess the activation status of immune cells. Samples were acquired using the Hyperion™ XTi Imaging System to capture both whole-tissue sections and subcellular-resolution regions of interest. CyTOF analysis revealed phenotypic and functional heterogeneity, including distinct T cell exhaustion and activation profiles. IMC analysis illuminated the spatial relationships of immune subsets relative to tumor cells, vasculature and stromal components, uncovering distinct patterns of immune infiltration and congregation. Merging CyTOF and IMC data revealed shared and divergent phenotypes between peripheral and tissue-resident immune compartments, highlighting the power of an integrated mass cytometry approach to identify potential biomarkers linking systemic immune profiles and the spatial heterogeneity of the TME.

Th182. Testing of Multi-Omics System Vaccinology Approaches on Pertussis Vaccine Dataset

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Abstract Text: New algorithms MOFA and Stabl represent approaches that resolve issues in analyzing complex, heterogeneous datasets by isolating key biological features that explain variability and outcomes of subjects in a

dataset. In this project, MOFA and Stabl were applied to data for pertussis vaccines (CMI-PB dataset) to predict the rankings of values for biological features at specific timepoints post-booster vaccination in 2023, using data from 2020-2022. The datasets included cell frequency, gene expression, cytokine and chemokine concentrations, antigen-specific antibody measurements, and T cell polarization and activation. MOFA used dimensionality reduction and ignored missing values to compute latent factors with weighted features, while Stabl filtered features with high missing values, implementing a KNN imputer before finding the most prominent features across models with different regularization strengths. Both effectively predicted IgG antibody levels against pertussis toxin on Day 14 with MOFA (Spearman correlation $\rho = 0.387$) identifying the baseline value, HSP90AB1, and RIPOR2 and Stabl ($\rho = 0.384$) using CHI3L2, USF1, and interleukin-6. Additionally, Stabl ($\rho = 0.172$) performed especially well in predicting frequency of Monocytes on Day 1, with monocytes and classical monocytes. Finally, MOFA ($\rho = 0.371$) excelled on the difficult task of predicting Th1/Th2 (IFN-γ/IL-5) polarization ratio on Day 30, leveraging the baseline value, IgG against filamentous hemagglutinin (both of which Stabl highlighted as well), and interleukin-17A. Overall, our exploration provides guidance and displays the benefits of using MOFA and Stabl to find the best predictive cell subsets and features for understanding large immunological multi-omics data.

Th188. Relationship of Early Farm Exposure on the Trajectory of Innate Immune Cell Function and Gut Microbiome

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Abstract Text: A protective association between early life animal farming exposure and development of allergic disease has been widely reported. Several reports have demonstrated increased pro-inflammatory blood immune features in farm-exposed newborns. The cell origins of the pro-inflammatory signals, the trajectory of immune phenotypes in early life, or associations with gut microbial communities in early life farm-exposed children has not been studied. Established in 2013, the Wisconsin Infant Study Cohort (WISC) is an ongoing birth cohort designed to further define the impact of early life farm exposure on immune development. Early life innate immune cell functions were measured by stimulating freshly isolated blood mononuclear cells with a panel of innate TLR agonists in animal farm exposed (n=73) and rural non-farm (n=96) children from birth to age 2. Flow cytometry and bead-based assays were used for immunophenotyping. Additionally, 16S rRNA sequencing was performed on serially collected stool samples within the first year. Mixed effects models were used to assess the trajectory of immune and microbiome variables. Farm-exposed children had significantly increased LPS-specific responses (myeloid dendritic cell intracellular TNF (p=0.015), secreted IL10 (p=0.007) and MIP-1b (p=0.018)) compared to non-farm children during the first 2 years of life. The gut microbiomes of farm-exposed children were more diverse than non-farm children (p=0.012), especially at nine months of age. Random forest analysis identified farm-predictive microbes, which included short chain fatty acid producers. These data show that farm-exposed children have a LPS-specific pro-inflammatory and regulatory immune trajectory in early life, alongside an increased rate of gut microbiome diversification.

Th189. Seromic Atlas of APS-1 Syndrome

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Abstract Text: Introduction: Recent advances in synthetic biology tools enable massively multiplexed serologic antibody profiling by phage display immunoprecipitation (PhIP-Seq) and protein microarray. We apply these tools toward unbiased investigation of antibody repertoires in APS-1 patients (autoimmune polyendocrine syndrome type

1). These patients bear widespread autoantibodies due to a genetic defect in the thymic transcription factor AIRE. Methods: To determine antibody repertoires in APS-1, we analyzed single or multi-timepoint serum samples (N=96) from over 70 unique APS-1 patients. Patients with diverse comorbidities were analyzed using human proteome microarrays (HuProt, >21,000 proteins), human proteome PhIP-Seq (HuScan, >700,000 49-mer peptides), and pan-viral PhIP-Seq (VirScan, >480,000 62-mer peptides). Patients negative for HSV-1 were additionally investigated using virion display arrays (VirD, >400 GPCRs and other membrane receptors). Results: In addition to previously reported autoantibodies against type I interferons, we observed numerous potentially novel autoantibodies associated with comorbidities such as diabetes mellitus, Sjögren's-like syndrome, enamel hypoplasia, and many others. Non-disease factors such as patient sex imparted additional features across the serologic profiles. In some patients, we detected autoantibodies against untagged membrane-embedded receptors on the virion display arrays. Conclusion: Many chronic conditions associated with APS-1 syndrome are found throughout the general population. This comprehensive autoantibody survey reveals potentially pathogenic mechanisms that, once validated, could inform therapeutic strategies for both APS-1 patients and the broader population affected by these conditions.

Th190. Single Cell Spatial Transcriptomics of the Human Bone Marrow Reveals Active Adaptive Immunity in Myelodysplastic Syndromes

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Abstract Text: Myelodysplastic syndromes (MDS) are malignant myeloid neoplasms defined by dysplastic growth of bone marrow populations, impaired hematopoiesis, and potential for progression to acute leukemia. While hematopoietic stem cell-intrinsic mechanisms of MDS have been rigorously analyzed, less is known about the role of the bone marrow microenvironment and adaptive immunity. To comprehensively study the human MDS microenvironment including the in situ distribution of immune populations, we built a custom targeted gene panel for spatial transcriptomic analysis of fixed, decalcified human marrow core biopsies (11 MDS, 5 controls, > 1.85x10^6 cells) paired with 5' single-cell CITE and T cell receptor (scTCR) sequencing of 58 marrow aspirates (35 MDS patients/8 controls, >6x10^5 cells and >1.55x10^5 TCRs). Nearest neighbor analyses revealed altered cell-cell relations in the MDS marrow including increased proximity of T regulatory cells to mature B cells and cytotoxic T cells, evidence for large immune aggregates (clusters of B cells, plasmacytoid dendritic cells, and T cells) reminiscent of tertiary lymphoid structures, and enrichment of hematopoietic stem and progenitor cells within these aggregates. T cells within immune aggregates had distinct phenotypes compared to those outside in MDS but not controls, including abundant memory T cells with stemlike features ($TCF7^+$). scTCR identified dominant $GZMB^+$ memory CD8 clones in MDS compared to controls and we discovered the in situ marrow distribution of dominant TCRs via creation of clone-specific probes for paired TCR-alpha/-beta sequences. Taken together, this study reveals the distinct T cell architecture of the MDS bone marrow microenvironment and supports a role for active T cell immunity in the MDS marrow.

Th191. Single-Cell Profiling of Circulating Monocytes Reveals Novel Platelet-Monocyte Complexes in HIV-Associated Atherosclerosis

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Abstract Text: Individuals living with HIV (PLWH) have an increased risk of developing atherosclerosis (AS), even when receiving antiretroviral therapy (ART). This risk arises partly from monocytes, which release pro-inflammatory cytokines, drive immune activation, and contribute to thrombosis. Notably, persistent platelet and monocyte activation in HIV leads to intensified interactions between these two cell types, further promoting inflammatory cascades and immune dysregulation in monocytes. In this study, we profiled 138,487 monocytes at single-cell resolution across four groups (HIV, AS only, HIV-AS double positive, and control) to investigate the heterogeneity of circulating monocytes. Our analysis revealed eight distinct transcriptional subpopulations, including CD14, CD14-active, CD16, Intermediate, Intermediate-metabolic, Intermediate-interferon monocytes, as well as two novel subsets: platelet-monocyte complexes and NK/proinflammatory monocytes. Furthermore, we applied a novel Gene-Set Analysis approach, single-cell Pathway Score (scPS), developed by our group to estimate disease-relevant pathways and transcription factors uniquely dysregulated in HIV-associated AS. These findings were reinforced by mass spectrometry-based proteomics and co-culture experiments, highlighting the central role of platelet-monocyte complexes in platelet activation and chemokine-driven signaling. Overall, this study reveals previously uncharacterized monocyte subsets and molecular signatures, thereby providing new insights into the mechanisms linking HIV and atherosclerosis, and identifying potential avenues for targeted therapeutic intervention.

Th195. Stabl Pairwise Statistic – a Flexible Approach to Feature Selection in Omics with Relaxed Exchangeability Assumption

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Abstract Text: Background: Omics technologies enable the measurement of thousands of features per sample, offering unprecedented resolution. However, identifying predictive features for clinical outcomes remains challenging. The Stabl framework [1] addresses this by injecting artificial noise to estimate the False Discovery Rate (FDR) but assumes global feature exchangeability, which is problematic given the variability in omics data distributions. We propose the Pairwise Statistic (PS), which relaxes this assumption by requiring only pairwise exchangeability. Methods: The PS quantifies the difference in selection frequency between a feature and its artificial counterpart. Assuming pairwise exchangeability, the distribution of non-predictive features aligns closely with their artificial counterparts relative to the clinical outcome. The optimal feature selection threshold is determined by minimizing a revised FDP^+, calculated as the ratio of positive PS above the threshold to negative PS below it. Performance was evaluated using Monte Carlo cross-validation (5 folds, 5 repeats) on COVID-19 and Onset of Labor (OOL) datasets and compared to the original Stabl. Results: The PS achieved predictive accuracy comparable to Stabl (COVID-19: 0.85 [0.75–0.94] vs. 0.79 [0.68–0.90]; OOL: 17.8 [14.3–21.6] vs. 17.8 [14.6–21.3]). Feature overlap between methods was high, with 3 of 4 features overlapping for COVID-19 and 18 of 19 for OOL. Conclusion: The PS provides a robust alternative to Stabl by relaxing assumptions of global exchangeability, achieving comparable predictive performance and identifying overlapping feature sets. It offers a flexible and effective approach for omics-based feature selection and biomarker discovery. [1] Hédou, J., Marić, I., Bellan, G. et al. Nat Biotechnol (2024). https://doi.org/10.1038/s41587-023-02033-x

Tu202. Systems Immunology Framework and Centrality Metric Provide Improved Insight into Immune Aging

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Abstract Text: The Immune System is a complex “Network of Networks”, in which various immune cell subsets interact and influence each other's functions, ultimately determine immune competence and the control or onset of disease. The coordinated interactions between these cell subsets determine whether the outcome is a normal physiological state or pathological condition. Established statistical procedures largely ignore the interactions between subsets and rely on statistically significant changes in the frequency of cell subsets. We developed CyNET (Cytometry Network)— an analysis platform based on a network science approach to understand the immune system holistically. CyNET enables us to quantify the systems level and subsets level properties. We used CyNET to analyze the immune development along the chronological age gradient. Peripheral blood cells from healthy newborns (cord blood), adults (20 to 55 years), and elderly (70 years and above) human subjects were analysed using CyNET and scRNA sequencing. We show that changes in the centrality of the nodes (immune subsets) reflect better biological functions than changes in frequency alone. For e.g. frequency of CD28-CD8 T cells increases with aging; however, our findings demonstrated reduced centrality and diminished ligand-receptor mediated intracellular interaction potential explaining senescence and exhaustion of these cells. Furthermore, we found systems property such as network edge density, degree centralization, and assortativity score reflect the maturation and development of the immune system along the age axis, thus enabling the characterisation of the functional architecture of the Immunome and the identification of key functional hubs within the immune networks along the age axis.

Tu204. The Epigenomic Landscape of CD4 T Cells in Relapsing-remitting Multiple Sclerosis Reveals Drivers of Disease Flare

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Abstract Text: Multiple sclerosis (MS) is an autoimmune disease characterized by the destruction of oligodendrocytes in the central nervous system, leading to progressive neurological impairment. The relapsing-remitting form of MS (RRMS) is marked by cycles of inflammatory attacks (flares) and remission periods, driven by $CD4^+$ T cells. While the role of these immune cells is established, the underlying epigenetic changes triggering disease flares remain poorly understood. To address this, we performed the first comprehensive cross-sectional ATAC-seq analysis comparing the $CD4^+$ T cell epigenomes during RRMS flares and remission. Our integrated approach combined proximal gene assignment and distal regulatory target identification through published cell type-specific Promoter Capture Hi-C data. This provided a more complete view of the disease-relevant genes impacted by dynamic chromatin accessibility changes. The cross-sectional analysis revealed a genome-wide reduction in chromatin accessibility during flares, particularly affecting anti-inflammatory programs, while pro-inflammatory pathways showed increased activity. Overlapping the differentially accessible regulatory regions (DAR CREs) with MS-associated genetic risk variants helped prioritize the most promising therapeutic targets. Importantly, Rummagen gene set analysis confirmed significant overlap between our identified DAR CRE networks and previously reported disease-specific and cell type-specific gene signatures. Moreover, our STRING network analysis uncovered novel target genes that showed direct protein-protein interactions with known MS regulators, suggesting their potential as previously unidentified therapeutic candidates. This cross-sectional epigenomic comparison between RRMS flares and remission offers critical mechanistic insights and highlights both validated and novel regulatory hubs as avenues for future therapeutic intervention.

Tu205. Using a Consensus Gene Signature to Systematically Annotate Exhausted T Cells

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Abstract Text: The exhaustion of T cells is prompted by chronic antigen stimulation and is characterized by terminal loss of effector T cell capabilities. T cell exhaustion has been mostly studied in the context of chronic inflammation and cancers, where exhaustion is pathogenic due to defective T cell immunosurveillance function. Recently, studies have highlighted T cell exhaustion in the context of autoimmune conditions, demonstrating that exhaustion is associated with increased flare-free survival time and slower disease progression. A challenge in studying the process of T cell exhaustion is the lack of unified approach of annotating the T cell exhausted state. We have curated a list of T cell exhaustion marker genes and used SARGENT, a gene-signature cluster-free based approach, to annotate T cells in publicly available cancer and immune-diseases datasets. We next used this approach to annotate T cells that have been exhausted using a re-stimulation by CD3/CD28. *In vitro* stimulation of T cells led to an increase in exhausted T cells. Moreover, multiple stimulations increased the proportion of exhausted T cells, suggesting that our annotation strategy accurately highlights exhausted T cells. Taken together, our unified annotation approach and an established T cell exhaustion assay will allow us to investigate the dynamics of T cell exhaustion and derive conclusions that could be translated to disease datasets.

Tu206. A Multi-tiered Statistical Framework for Characterizing Morphological Cell-to-cell Interactions in Spatial Transcriptomics

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Abstract Text: In spatial transcriptomics, cell-to-cell interactions are critically important because they influence various biological processes and phenomena that are central to understanding tissue function and disease. Single-cell-level profiling in spatial transcriptomics sheds light on the heterogeneity and dynamics of individual cells. This study aims to establish a statistical pipeline for identifying regions of unusual activity in cell frequency and characterizing morphological cell-to-cell interactions in those regions, which are vital for immune responses. We begin by annotating cells based on their gene expression profiles and selecting two cell types of interest for further analysis. The spatial domain is divided into subdomains based on a biological length scale, defining a meaningful size for each subdomain (i.e., niches). In each subdomain, we apply a first-order statistic to assess the frequency of each cell type and identify regions with abnormal cell-type distribution. These subdomains are further analyzed using a second-order statistic to examine cell-to-cell spatial distances, potentially indicating local signaling interactions between the selected cell types. If unusual spatial interactions are detected, we use a third-order statistic to explore detailed collocational alignments between the two cell types. This multi-tiered approach provides a comprehensive view of how cell interactions are spatially organized and how localized environments influence tissue behavior. Ultimately, this method has the potential to uncover insights into tissue development, disease mechanisms, and therapeutic strategies by focusing on the spatial organization of cellular interactions. This framework is applied to spatial data from psoriasis skin biopsies in the PAUSE trial at the Immune Tolerance Network (ITN).

Tu207. Batch Effect Reduction Promotes Pattern Discovery in High-dimensional Mass Cytometry Data

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Abstract Text: Mass cytometry (MC) measures over 50 proteins in single cells and enables profiling of immune cell composition. Machine learning and statistical methods identify cell types and interrogate their correlation with physiological conditions like disease or age. However, fluctuations in instrument readouts, called batch effects, can interfere with detecting critical biological variations. In a previous report on the EPIC (Extended Polydimensional

Immunome Characterization) data mining platform, we showed that batch-wise scaling effectively reduced batch effects in MC data. To visualize and quantify the impact of batch effects on sample stratification, we developed group similarity analysis (GSA). This technique combines dimension reduction (e.g., UMAP) with silhouette analysis to find patterns in summary statistics of clustering outputs. We used GSA to compare batch scaling with six other batch normalization algorithms. Using a dataset of 159 PBMC samples of healthy donors acquired in 35 MC runs, we show that batch normalization improves stratification into three age groups. A second case study of 153 samples from liver cancer patients demonstrates that batch normalization improves the segregation of cell composition according to tissue types (peripheral blood, liver). The conclusions from GSA agree with other methods for evaluating batch alignments, such as Earth Mover's (EMD) or the coefficient of variation among bridging replicates. In summary, batch effect reduction promotes biological pattern discovery in cytometry data.

Tu208. Characterising Drivers of Human Immune Variation Using High Content High Throughput Cytometry and Machine Learning

Tom Hayday, Jack Bibby, Eduardo de Paiva Alves, Gabija Drazdauskaitė, Hamed Haseli Mashhadi, Adam Laing, Jeremy Mason, Duncan McKenzie, Benjamin Thomas, Pelle Ullberg and Jennie Yang

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Abstract Text: Immune system variation underpins diverse responses to therapies, vaccinations, and disease, yet remains poorly characterised across healthy populations. To address this gap, we conducted a comprehensive immune profiling study of 1,009 predominantly healthy individuals, integrating high-resolution spectral flow cytometry data with clinical blood biochemistry and extensive health and lifestyle questionnaires. Our novel machine learning (ML) platform provides real-time immune cell classification from spectral flow cytometry data, facilitating standardised, robust and reproducible characterization of over 2000 immune cell subsets. Key findings from the study include age- and sex-associated immune signatures, revealing novel insights into immune aging and sexually dimorphic immune profiles. Further, by correlating immune phenotypes with clinical parameters, we identified hundreds of immunome-wide associations linking immune variation to health status and lifestyle factors. The platform's scalability, enabled by industrialised sample processing and ML-driven analytics, addresses a critical need for population-scale immune profiling. The ability to normalise and interpret data from cryopreserved samples ensures compatibility with large biobanks. This capability sets the foundation for integrating immune data into broader clinical and genetic datasets to unlock new perspectives on human health and disease. This study underscores the transformative potential of next-generation flow cytometry combined with ML analytics to generate high resolution immune profiling data at population-scale to enable precision immune medicine, patient stratification, and biomarker discovery.

Tu209. Common and Divergent Dynamic Changes of the Human Plasma Metabolome Across the First Week of Life in the Gambia and Papua New Guinea

Annmarie Hoch[1], Caitlin Syphurs[1], Jing Chen[1], Rym Ben-Othman[2], Asimenia Angelidou[1], Kinga Smolen[1], Oludare Odumade[1], Kerry McEnaney[1], Olubukola Idoko[3], Geraldine Masiria[4], Joe Jude[4], Rebeca Ford[4], Jessica Lasky-Su[5], Al Ozonoff[1], Anita van den Biggelaar[6], Peter Richmond[7], William Pomat[4], Beate Kampmann[8], Tobias Kollmann[7], Ofer Levy[1] and Joann Arce[1]

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Abstract Text: Background: The plasma metabolome changes markedly over the first week of life yet little is known regarding how these trajectories might differ across geographically distinct populations. Methods: To gain insight into potential differences in ontogeny between geographic sites, newborn participants were enrolled in The Gambia (n=45) and Papua New Guinea (n=45). All participants had samples collected at birth and were randomized to have their second sample collected either on the first, third, or seventh day following birth. Global untargeted plasma metabolomics employed ultra-performance liquid chromatography mass spectrometry (Metabolon). Findings: PCA showed plasma metabolites clustering by day of sample collection and by site. The majority (62%) of metabolite features showed a significant difference based on time point: 46% of these were lipids and 17% were amino acids. Additionally, 24% of the metabolite features showed a significant difference between sites, with the most common being lipids (28.2%). Xenobiotics including caffeine and food metabolites were distinct in GAM vs. PNG, suggesting differences in maternal diet and environmental factors. Conclusions: The plasma metabolome undergoes marked changes during the first week of human life, with distinct features across geographic locations, and warrants further exploration with respect to potential correlations to immune status and clinical outcomes, with particular emphasis on lipid pathways.

Tu210. Deciphering Immune Cell Drivers of Health and Disease Through the Lens of a 40M Blood Cell Atlas

Jacquelyn Nestor[1], Wamia Said[1], Sergio Aguilar Fernandez[1], Pragya Rawat[1], Roya Best[1], Adrien Antoinette[1], Rachelly Normand[1], Nandini Samanta[1], Sidney Martin[1], Hoang Anh Tran[1], Kamil Slowikowski[1], Neal Smith[1], Thomas Chen[1], Isabela Kernin[1], Courtney Ambrose[1], Alice Tirard[1], Kian Hong Kock[2], Michaela Mueller[3], Ana-Maria Cujba[4], Dejan Juric[1], Ryan Sullivan[1], Genevieve Boland[1], John Stone[1], Eilish Dillon[5], Devin King[5], April Jorge[1], Andrew Luster[1], Kerry Reynolds[1], Maureen Leonard[1], Tanuja Chitnis[5], Malte Luecken[3], Kevin Wei[5], Deepak Rao[5], Michelle Rengarajan[1], Pritha Sen[5], Nir Hacohen[1], Holger Heyn[6], Shyam Prabhakar[2], Gary Reynolds[1] and Alexandra-Chloé Villani[1]

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Abstract Text: Blood analysis is an essential clinical tool, providing an accessible window into human health. Broad shifts in major immune cell lineages in blood can be critical diagnostics; however, deeper profiling may uncover nuanced shifts in smaller subsets, revealing further clues to diagnosis, prognosis, disease manifestations and even treatment response. This level of granularity has the potential to redefine the clinical complete blood cell counts and identify cellular and biological programs that are shared and distinct across a range of immune disorders that could have therapeutic potential, setting the foundation towards developing precision medicine approaches for immune-mediated diseases. We employed single-cell multiomics technologies and generated an internal dataset across patients with checkpoint-inhibitor toxicity, infections, and autoimmune conditions. This internal dataset in combination with multiple external datasets, will culminate in a substantial dataset of ~10,000 donors and over 40M cells across 40 conditions. A dataset of this size will allow for mapping of all circulating cell subsets with a frequency of 0.001%. Downstream analysis of this unique dataset and immune cell subsets identified will explore differential abundance, transcriptional programs, localization of GWAS risk variants and immunoassays assessing cell functionality. Our preliminary analysis reassuringly recapitulates known biology, while also identifying novel immune cell subpopulations related to specific diseases. An Immune Cell Atlas of this size will not only define the wide spectrum of immune cell diversity across health and disease, but will undoubtedly be critical to expanding our knowledge of cross-disease immune cell programs, while simultaneously highlighting disease-specific variations.

Tu211. Defining Spatial Signature of TNF Inhibition in Ulcerative Colitis with a Deep Learning-Based Algorithm for Case-Control Analysis

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Abstract Text: The heterogeneity of autoimmune diseases makes it challenging to characterize general molecular mechanisms or predict optimal personalized treatments. Even predicting the direct molecular effects of treatment has proven difficult, with many single-cell studies failing to stratify patients by treatment. Spatial -omics data can link molecular- to tissue-scale phenotypes, but most methods for analyzing spatial data require tissue annotations that ignore local spatial relationships and/or require error-prone cell segmentation, limiting signal strength. To overcome these limitations, we applied Variational Inference-based Microniche Analysis (VIMA), our new deep learning-based method for case-control analysis of spatial data, to a large tissue atlas of ulcerative colitis. Through analysis of 52 surface protein markers from the sub-epithelial layers of 42 colonic biopsies, we found significant differences in protein expression and spatial distribution between patients treated with TNF-inhibitors (TNFi) and untreated patients (P=6.5e-4). VIMA identified spatial niches that covaried with TNF treatment duration, stratifying samples from TNFi-naive, through recent TNFi initiation and prior TNFi use, to longtime TNFi treatment. TNFi-associated tissue niches displayed disorganized tissue architecture while TNFi-naive-associated niches contained highly organized, B-cell-dominated lymphoid aggregates. TNFi-associated niches also had lower lymphoid marker levels and higher levels of fibroblast, macrophage, and mesenchymal markers, indicating treatment-associated cell-type-specific tissue infiltration. Intriguingly, TNFi-discontinued samples resembled those from patients currently receiving TNFi, suggesting long-term tissue remodeling induced by TNF inhibition. These results highlight VIMA's unique ability to isolate subtle spatial signals, shed light on the molecular consequences of TNF inhibition, and expand our understanding of variable responses to TNFi.

Tu212. Drivers of Differential Antibody Breadth in SARS-CoV-2 mRNA Vaccination and Infection

Prasanti Kotagiri[1], Oliver Wirz[2], Emily Haraguchi[2], Katharina Röltgen[2], Molly Hunter[3], Erin Craig[2], Jumana Afaghani[2], Ji-Yeun Lee[2], Sandra Nielsen[2], Ramona Hoh[2], Taylor Pursell[2], Prabhu Arunachalam[2], Monali Manohar[2], Iris Chang[2], Jackson Schuetz[2], Brandon Lam[2], Andrea Fernandes[2], Evan Do[2], Donna Smith[4], Brian Ha[4], Linda Liao[5], Chandler Ho[5], Jacob Wohlstadter[6], George Sigal[6], Stuart Scott[2], Sean Van Slyck[4], Mark Davis[2], Bali Pulendran[2], Jeremy Minshull[3], Benjamin Pinsky[2], Kari Nadeau[7], Claus Niemann[8], Fan Yang[2] and Scott Boyd[2]

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Abstract Text: The cellular, immunogenetic and antigenic factors that affect the breadth of viral antigen variants recognized by human antibody responses are poorly defined. mRNA vaccination for SARS-CoV-2 (CoV-2) results in initial polyclonal antibody mixtures of greater breadth compared to those stimulated by viral infection. Publicly available CoV-2 binding antibodies characterized during the pandemic are relatively few in number, biased toward neutralizing antibodies, and characterized for binding to few variants, primarily due to the expense and labor required for monoclonal antibody production/characterization. To markedly scale up human antibody analysis to address difficult questions such as the molecular determinants of serological breadth, we have developed highly multiplexed panels of DNA-tagged CoV-2 antigens from up to 20 viral variants to label, sort and enable B cell receptor and transcriptome sequencing of 6,262 antigen-binding B cells from peripheral blood of mRNA vaccinees, infected patients and lymphoid tissues of deceased organ donors. Antigen-specific B cells were enriched in a circulating class-switched memory subset with evidence of recent germinal center exposure and progressive somatic hypermutation over time, and atypical B cells that had minimal mutation accumulation. Surprisingly, mRNA vaccination preferentially stimulates B cells expressing B cell receptors with inherently high antigen binding breadth, compared to infection-stimulated B cell clones. The results from this scaled-up analysis enable systematic modeling of the B cell subset, antigen exposure and BCR composition determinants of antigen binding breadth, a topic of increasing importance as mRNA vaccination is evaluated for other viruses with high mutation rates contributing to evasion of human antibody responses.

Tu213. Floren: A Combination of Heterogeneous Graph Transformer and Graph Self-supervised Learning to Analyze Highly Specific Patterns in Single-cell Transcriptomic Data

Iñigo Clemente-Larramendi[1], Divi Cornec[2], Nathan Foulquier[1], Sophie Hillion[1] and Christophe Jamin[1]

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Abstract Text: Single-cell transcriptomics has emerged as a powerful technique, with increasing data collection and complex experimental designs necessitating more flexible and accurate analytical methods. Graph Neural Networks (GNNs) have shown promise in this domain, with over 40 tools published in the past four years. However, most focus on homogeneous graph learning for cell annotation or clustering. To our knowledge, only DeepMAPS employs heterogeneous graph learning, and only Cellograph analyzes single-cell transcriptomic data at the differential gene expression (DGE) level. We present FloREN, a GNN pipeline that extends DeepMAPS to multiple samples and feature connections, enhancing the Cellograph approach to address not only DGE but also transcription factors (TFs), cell states, regulatory pathways, and more. Our method integrates an autoencoder (AE) to establish a common representation for cells and genes, applies Heterogeneous Graph Transformers (HGT) to learn informative networks per sample, and utilizes a modified Graph Contrastive Learning (graphCL) technique to extract group-relevant patterns. We demonstrate FloREN's learning capabilities and flexibility on approximately 2 million cells from around 400 patients across four autoimmune conditions.

W103. An Automated and Scalable Pipeline for High-dimensional Immune Cell Phenotyping in Mass Cytometry Datasets

Julien Hédou[1], Grégoire Bellan[1], Xavier Durand[1], Alexandre Maillard[1], Franck Verdonk[2], Brice Gaudillière[3], Dominique Blanchard[1] and Benjamin Waked[1]

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Abstract Text: Context: Efficient single-cell phenotyping has become essential for the identification of complex cell populations. Traditionally, these populations are identified through manual gating, a time-consuming process, subject to variability and a lack of reproducibility. Here, we adapted a deep learning model to devise an automated gating pipeline with the goal of enhancing the accuracy, speed, and reproducibility of immune cell gating. Methods: Our pipeline was evaluated on two mass-cytometry datasets (>5M cells, 35 markers), that had been previously gated by experts for comparison purposes. Phenotypic markers were used for identification, while the median expression of intracellular markers was used to determine the functional properties of populations. Results: The automated gating pipeline achieved high overall accuracy comparable to expert manual gating (n=30 cell populations) (0.917 and 0.921 for dataset 1 and 2, respectively). Processing time was significantly reduced: < 12min for both datasets (8CPUs, 8GB of RAM). High-level populations (n=10) were identified with excellent accuracy (e.g. T cells, Bcells with f1-score of 0.993, 0.937 on dataset 1 and 0.996, 0.934 on dataset 2). However, rare populations (n=20) showed higher discrepancies (e.g. intermediate monocytes, DC with f1-scores of 0.864, 0.816 and 0.678, 0.552 on dataset 1 and 2, respectively). Differences in identification scores had low effect on the functional properties of populations, as 91.4% of the functional properties defined from our pipeline were highly correlated (r>0.75) with those derived from manual gating. Conclusion: This automated approach eliminates operator biases and handles multiple markers simultaneously, offering reliable and efficient analyses for research and clinical applications.

W212. Generation of Synthetic Single Cell Transcriptomic Data from High-throughput Cytometry Enables Population-scale, Deep Resolution Immunophenotyping in Health and Disease

Duncan McKenzie, Eduardo de Paiva Alves, Jack Bibby, Hamed Haseli Mashhadi, Lilian Williams, Sergii Gryshkevych, Benjamin Thomas, Jennie Yang, Tom Hayday, Jeremy Mason and Adam Laing

IMU Biosciences, London, England, United Kingdom

Abstract Text: Single cell transcriptomics has driven the rapid progress of systems immunology by revealing the heterogeneity and complexity of the immune system with unprecedented resolution. However, broad clinical impact has been constrained owing to limitations in throughput, excessive cost, and significant sampling error and reproducibility associated with low cell numbers captured per sample. To address this, IMU Biosciences has developed Transcriptomic-Enhanced Cellular High-throughput Phenotyping (TECH-P); a novel machine-learning (ML) approach that uses high-content spectral flow cytometry to faithfully produce synthetic single cell transcriptomic data. Trained on a growing set of samples from healthy donors and patients processed in parallel by the IMU phenotyping platform and single cell RNA sequencing, TECH-P employs modality harmonisation alongside deep learning to accurately predict gene expression profiles for hundreds of thousands of cells per sample. Application of TECH-P to an internal dataset of 1,048 individuals profiled by the IMU platform generated a single cell atlas of the healthy immune system comprising ~120 million cells, surpassing extant single cell atlases. This largescale synthetic immune database faithfully describes known cell subset specific transcriptional programs and replicates known single cell transcriptional signatures associated with aging. Ongoing development of TECH-P aims to bridge single-cell transcriptomics with routine, high-throughput and statistically robust immune profiling of clinical samples. This integration has the potential to transform a wide range of applications, from target discovery and drug development to patient stratification and precision medicine.

W213. High-Plex NULISAseq Inflammation Panel 250 AQ for Comprehensive Profiling of Immune and Inflammatory Biomarkers with Absolute Quantification

Xiao-Jun Ma, Chan Ho Park, Alissa O', Connor, Tianyang Evan Bai, Cheng Xie, Joanne Beer, Niyati Jhaveri, Dwight Kuo, Bingqing Zhang, Yiyuan Yin and Yuling Luo

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Abstract Text: Highly multiplexed profiling of biomarkers associated with inflammatory, autoimmune and infectious diseases can aid in identification of new diagnostic and therapeutic strategies. Previously, we developed the NULISAseq Inflammation Panel 250-plex assay on the Argo HT platform for relative quantitation of key cytokines, chemokines, and proteins involved in inflammation and immune response. Absolute quantification (AQ) of analytes in mass concentration units (pg/mL) can be valuable in translational and clinical settings for biomarker validation, disease stratification and monitoring of treatment responses. However, conventional immunoassay quantification involves constructing multi-point calibration curves for each analyte, reducing available wells on the assay plate for samples and adding costs. To address this challenge, we developed a novel approach requiring a single calibrator sample to derive absolute concentrations of samples using a proprietary algorithm. Here, we report analytical characterization of the Inflammation Panel 250 AQ assay, enabling AQ of >150 analytes simultaneously with minimal sample input and high throughput. Precision and reproducibility assessments demonstrated 5.75% intra-plate and 2.86% inter-plate median CV across 156 AQ targets. With variance component analysis, total median CV was 8.35% across different reagent lots and instruments. Limits of quantification determination demonstrated a wide dynamic range with 11.4 logs across all targets and up to 6 logs for individual targets. Additionally, >90% of targets showed >50% detectability and >50% quantifiability in a cohort of healthy and diseased plasma (n=119) and serum (n=53) samples. In summary, the NULISAseq Inflammation Panel AQ provides absolute quantification of >150 analytes to enable discovery of key targets for immunology research.

W214. Immunology-driven Semi-automatic Annotation of Single-cell Data Using Machine Learning Approaches

Adrien Antoinette[1], Courtney Ambrose[1], Roya Best[1], Genevieve Boland[1], Thomas Chen[1], Tanuja Chitnis[2], Ana-Maria Cujba[3], Eilish Dillon[2], Nir Hacohen[1], Holger Heyn[4], Dejan Juric[1], Isabela Kernin[1], Devin King[2], Kian Hong Kock[5], Maureen Leonard[1], Malte Luecken[6], Andrew Luster[1], Sidney Martin[1], Michaela Mueller[6], Jacquelyn Nestor[1], Rachelly Normand[1], Shyam Prabhakar[5], Deepak Rao[2], Pragya Rawat[1], Michelle Rengarajan[1], Gary Reynolds[1], Kerry Reynolds[1], Wamia Said[1], Nandini Samanta[1], Pritha Sen[7], Kamil Slowikowski[1], Neal Smith[1], John Stone[1], Ryan Sullivan[1], Alice Tirard[1], Hoang Anh Tran[1], Alexandra-Chloé Villani[1], Kevin Wei[2] and Sergio Aguilar Fernandez[1]

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Abstract Text: The need for a unified system of cellular nomenclature has driven the development of single-cell atlases. To be truly comprehensive these must encompass millions of cells and incorporate cellular diversity induced by a range of disease perturbations. However, challenges such as non-biological variation and inconsistent sample metadata hinder their construction, requiring optimized data wrangling and integration workflows. As the scale of these datasets grows, cell-type annotation becomes increasingly labor-intensive, with automated tools often struggling to balance accuracy and adaptability. To address these challenges, we assembled a dataset of 25 million cells spanning 40 diseases, integrating data from 20 public studies and in-house efforts, with contributions from over 1000 donors. This dataset represents a wide range of disease states, including autoimmune conditions (e.g., systemic lupus erythematosus, rheumatoid arthritis, IgG4-related disease), infections (e.g., Zika, viral hepatitis,

CMV, EBV), and iatrogenic causes (e.g., immune-checkpoint inhibitor toxicities). We benchmarked data harmonization and integration methods to retain biological signals. To support cellular annotation we developed a semi-automated framework employing a decision tree approach with XGBoost, combining gene and protein expression based on finely-annotated training data. This achieved a mean balanced accuracy of 0.85 across 22 cell types, with a run-time of under five minutes. This strategy significantly reduces annotation time while remaining versatile and adaptable to a wide range of contexts. This study offers a structured framework for constructing single-cell reference atlases, paired with an innovative methodology to expedite cell-type identification and a resource for understanding disease-associated cellular diversity.

W216. Integration of Systems Immunology and Serology to Decipher Maternal Predictors and Determinants of Antibody Transfer in Mother-infant Dyads

Can Liu[1], Andrew Martins[1], Ao Huang[1], Shoham Benmelech[1], Dhvanir Kansara[2], Pieter Pannus[3], Meirav Sela[1], Larissa Debrabandere[4], Isabelle Desombere[5], Leo Heyndrickx[6], Kevin Arien[6], Maria Goossens[5], Kirsten Maertens[4], Margaret Ackerman[2], Arnaud Marchant[3] and John Tsang[1]

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Abstract Text: While it is appreciated that pregnancy alters maternal immune states, major knowledge gaps remain. For example, how various immune cell types and their states are shifted throughout gestation, and crucially, how these changes might impact function such as responses to vaccines and subsequent antibody transfer to neonates, are not well understood. To address these gaps, we conducted a multi-center study involving two distinct maternal cohorts: one immunized with the BNT162b2 COVID-19 mRNA vaccine and the other with the influenza vaccine. Using a combined systems immunology and serology approach, we applied multiplexed CITE-seq and bead-based Ig and Fc antibody profiling to examine the immune cell and serological landscapes of pregnant and non-pregnant women before and after vaccination. Our findings revealed distinct immune profiles in pregnant women, marked by heightened IFN response signatures and unique shifts in cell states at baseline. We hypothesized that hormones are major regulators of maternal immune states and indeed, *in vitro* stimulation with different estrogen levels mirrored *in vivo* immune cell state shifts. By integrating single-cell multiomics data with system serology, we identified maternal predictors of vaccine responses and maternal-to-fetal antibody transfer distinct from non-pregnant women, thus suggesting pregnancy-specific predictors and determinants of vaccine responses. Related baseline states appear to predict the extent of antibody transfer to the infant beyond the magnitude of the maternal serological responses, especially for the mRNA vaccine. These findings advance both basic immunological insights and provide a foundation for optimizing maternal vaccination strategies to enhance maternal and infant immunity.

W217. MultiTIE: Sanofi's Multi-Specific Target Identification Engine

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Abstract Text: Modulating two or more disease-associated pathways simultaneously via multispecific therapy is a prominent drug development strategy. In drug development research, paired target selection is driven by combining proven monospecific compounds or logic-based selection from a small pool of literature-supported targets. Despite the high interest in machine-guided algorithms for unbiased decision-making, there are no data-driven methods to systematically evaluate multi-target hypotheses. To such end, Sanofi's R&D developed an end-to-end multispecific Target Identification Engine (MultiTIE) tuned to predict the efficacy of obligate multispecifics and

combination/bifunctional therapies. MultiTIE integrates disease tissue data from transcriptomics (bulk, single cell and spatial) and proteomics, genetics, and knowledge graphs. For each data type, MultiTIE includes novel "relational" metrics that measure how two genes are suitable to be targeted together. These metrics are then combined into a single score to rank millions of bispecific hypotheses against each other. Rigorous benchmarking against clinically validated bispecific pairs demonstrates that high MultiTIE scores are enriched for successful assets. The MultiTIE framework applies machine learning algorithms to learn and train on medium-sized functional screens and clinical successes. Finally, to enable rapid decision-making, MultiTIE integrates scientist-interpretable visualizations, such as cell type-specific functional pathway engagement predictions, anchor-based selection, and benchmarking. This effort is built upon agile development and FAIR design principles to enable a strong and durable Sanofi-learned proprietary engine with consistently evolving capacity. Since its initiation, MultiTIE has been developed for 10 high-priority indications impacting more than 10 pipeline decisions.

W218. Nasal Transcriptome Changes in Response to Acute Respiratory Viral Infections in Patients with Rheumatoid Arthritis

Gabrielle Smith-Rockne[1], Matthew Lawrance[1], Naresh Doni Jayavelu[1], Kimberly Dill-McFarland[2], Cate Speake[1], Jane Buckner[1], Matthew Altman[2] and Carmen Mikacenic[1]

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Abstract Text: Acute respiratory viral infections (ARVI) are the most frequently occurring global illnesses causing significant morbidity and mortality. Adults with rheumatoid arthritis (RA) have increased risk of infection and clinical complications, although the underlying molecular and cellular immune responses remain poorly understood. To address this, we examined transcriptional changes in the upper airway of RA (n=28) and healthy control (HC, n=13) participants during ARVI. We collected nasal swabs at multiple timepoints: Baseline, Days 1-3, Days 4-6, Days 8-11, and 30 days post-symptom onset, and one year post-baseline. Bulk RNA-sequencing was performed followed by differential gene expression analysis using a linear mixed effects model to identify genes with different temporal patterns through acute infection. The majority of participants were infected with SARS-CoV-2 (n=24). Considering airway transcriptomic changes, we observed minimal transcriptional differences between RA and HC patients at Baseline, Days 1-3, and Days 4-6, but there were a greater number of differentially expressed genes over time in RA (n=4102) compared to HC patients (n=324). Next, we built gene modules using Weighted Gene Co-expression Network Analysis (WGCNA) and found that interferon and adaptive immune response modules exhibited a similar pattern of increased expression over time in RA and HC. However, expression of modules enriched in cilium associated pathways and epithelial associated pathways changed exclusively in the RA cohort. Our novel longitudinal study suggests that while there are minimal baseline differences between RA and HC, ARVI induces more temporal transcriptional changes in the upper airway in RA, including alterations in ciliary and epithelial pathways.

W219. Powerful and Accurate Case-control Analysis of Spatially Resolved Molecular Data Using Deep Learning-defined Tissue Microniches

Yakir Reshef[1], Michelle Curtis[1], Anna Helena Jonsson[2], Ilya Korsunsky[1], Soumya Raychaudhuri[1], Laurie Rumker[1] and Lakshay Sood[1]

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Abstract Text: As spatial molecular data grow in scope and resolution, there is a pressing need to identify key spatial structures associated with disease. However, current approaches often rely on hand-crafted tissue features that may overlook informative signals. We introduce a method, variational inference-based microniche analysis (VIMA), that uses deep learning and principled statistics to discover disease-associated spatial structures with

greater flexibility and precision. VIMA uses a variational autoencoder to define a large number of “microniches” – small groups of tissue patches with highly similar biology spanning multiple samples – and identifies microniches whose abundance correlates significantly with disease. We show using simulations with real spatial transcriptomics datasets that VIMA is more powerful and accurate than simpler approaches. We applied VIMA to an N=27 immunohistochemistry dataset in rheumatoid arthritis (RA) and an N=75 spatial transcriptomics dataset of 140 genes in Alzheimer’s Disease. In the RA dataset, VIMA identified multiple spatial structures differentially enriched among disease subtypes previously defined using scRNA-seq (P=6.6e-4), including macrophages in contact with sublining fibroblasts, perivascular cellular infiltrates, highly vascularized regions, and CLIC5^{+} lining fibroblast-rich regions. In the Alzheimer’s dataset, VIMA revealed a previously undescribed positive association between dementia and an oligodendrocyte-rich niche in cortical layer 6, while recapitulating a known negative association between dementia and the thickness of cortical layers 2/3 (P=2.2e-3). These spatial features strongly separated dementia cases from controls, which was not possible using non-spatial sc-RNAseq. Our findings show the promise of spatial technologies for unraveling the complex tissue processes that typify autoimmune disease.

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