ABSTRACT SUPPLEMENT

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Allergy

Th100. Impact of Adjunct Therapies on Hospital Length of Stay in Pediatric Perioperative Anaphylaxis: A PHIS-Based Analysis

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Abstract Text: Background: Perioperative anaphylaxis is a life threatening condition in children, presenting with hypotension, bronchospasm, urticaria, and flushing. Epinephrine is the primary treatment. Therapies such as corticosteroids and antihistamines are administered despite limited evidence supporting better outcomes. Hospital length of stay serves as a key indicator of illness severity. This study evaluates the association between adjunct therapies and hospital LOS in pediatric patients with perioperative anaphylaxis. Methods: A retrospective cohort study using the Pediatric Health Information System (PHIS), an administrative database of over 40 U.S. tertiary children's hospitals, was conducted. Pediatric patients (0–18 years) diagnosed with perioperative anaphylaxis who received at least one dose of epinephrine between January 2010 and December 2020 were included. Patients were categorized into three groups, one with epinephrine only, a second with epinephrine and corticosteroids, and another with epinephrine, corticosteroids and antihistamines. Patients with incomplete medication data or who did not receive epinephrine were excluded. The primary outcome was hospital LOS, analyzed using the Kruskal-Wallis test. Results: A total of 1,113 patients met inclusion criteria. Mean LOS was 1.08 days for the epinephrine only group, 1.31 days for the epinephrine with corticosteroids, and 1.60 days when given three therapies. The difference in LOS between groups was statistically significant ($p < 0.01$). Conclusion: The addition of corticosteroids and antihistamines to epinephrine in pediatric perioperative anaphylaxis was associated with increased hospital length of stay. These findings highlight the need for further prospective studies to assess whether adjunct therapies improve outcomes or serve primarily as markers of illness severity.

Th101. Whole Blood Stimulation, Cytokine and HLA-Allele Profiling in Patients Presenting with Hypersensitivity to Titanium-Containing Orthopaedic Implants in Pretoria, South Africa

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Abstract Text: Background: Systemic metal hypersensitivity (SMH) is an increasingly recognised immune mediated complication associated with metallic orthopaedic implants, commonly driven by Type IV hypersensitivity reactions. While contact allergic reactions to metals (e.g. nickel) is well described, the systemic immunopathogenesis to titanium implants remains poorly understood. Presentations range from local to systemic manifestations, including persistent pain, implantation failure, eczematous eruptions, chronic pruritus, fatigue and unexplained inflammatory symptoms. Existing diagnostic approaches, particularly patch tests (PT), demonstrate suboptimal sensitivity for detecting titanium-related systemic hypersensitivity, highlighting the need for more robust immunologic biomarkers. In addition, PT are unreliable as screening tests. Accumulating evidence points to a role for dysregulated cytokine response and specific human leukocyte antigen (HLA) class II alleles in conferring susceptibility to SMH. However, studies integrating cytokine profiling, whole blood stimulation assays and HLA genotyping in patients with titanium orthopaedic implants are limited. Objectives: The main aim of this study is to characterize cytokine profiles after whole blood stimulation and HLA allele distributions in patients with titanium orthopaedic implants presenting with SMH. Detailed objectives include: I) assessing the immune response *in vitro* to titanium dioxide using whole blood stimulation assays; II) comparing cytokine signatures between symptomatic implant recipients, asymptomatic

implant recipients and healthy controls without implants; III) identifying HLA alleles associated with hypersensitivity reactions to titanium implants; IV) exploring associations between demographic, clinical, cytokine and HLA profiles as well as the presence and severity of hypersensitivity reactions. Methodology: This cross-sectional descriptive study is recruiting adults with orthopedic implants and SMH to titanium who test positive on a lymphocyte proliferation assay (n=30); implant recipients without hypersensitivity symptoms (n=30); and healthy controls without implants (n=20). Participants are recruited from orthopedic and allergy practices in Pretoria, South Africa. Whole blood samples are collected for *in vitro* stimulation with titanium dioxide and lipopolysaccharide as a control. Post-stimulation plasma cytokine levels are quantified using multiplex suspension bead array assays. HLA class II genotyping is performed using bead-based molecular assays. Clinical, demographic and laboratory data will be analyzed using multivariable logistic and linear regression models to identify independent predictors of hypersensitivity and disease severity.

Tu100. Signal-Transducing Adaptor Protein-1 Is Essential for Modification of FcepsironRI-mediated Mast Cell/Basophil Activation by Lyn/SHP-1 Interaction

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Abstract Text: Mast cells and basophils are critical cell types in allergic inflammatory reactions. Once IgE-bound mast cells and basophils are activated by multivalent antigens, the release of chemical mediators and the production of cytokines/chemokines are induced, leading to the initiation of allergic inflammation. The Signal-Transducing Adaptor Protein (STAP) family consists of two members, STAP-1 and STAP-2, both of which participate in immunological signal transduction. The role of STAP-2 is characterized by a distinct dichotomy; it selectively suppresses FcepsironRI signal transduction in mast cells but functions as an essential enhancer for FcepsironRI-mediated activation in basophils. However, the functional role of STAP-1 in mast cell/basophil activation remains unknown. In this study, we investigated the role of STAP-1 in FcepsironRI-mediated activation of mast cells and basophils. The number of mast cells in the back skin and the proportion of basophils in the blood of STAP-1-deficient (STAP-1 KO) mice were comparable to those of wild-type (WT) mice. Furthermore, the yields of bone marrow-derived mast cells (BMMCs) and bone marrow-derived basophils (BMBs) from STAP-1 KO mice were equivalent to those from WT mice, indicating that STAP-1 is dispensable for the generation of these cells. While antigen-induced degranulation in STAP-1 KO BMMCs was similar to that in WT BMMCs, cytokine production in activated STAP-1 KO BMMCs was significantly enhanced. We found that the phosphorylation of Lyn Y396 was increased in STAP-1 KO BMMCs compared with WT BMMCs. Immunoprecipitation experiments revealed that STAP-1 is required for the SHP-1 association with Lyn after FcepsironRI aggregation, as STAP-1 deficiency abolished the interaction between Lyn and SHP-1 in activated mast cells. Additionally, the IgE/Ag-induced production of IL-4, IL-6, and TNF in STAP-1 KO BMBs was significantly higher than the production in WT BMBs. Taken together, these results indicate that STAP-1 acts as a negative regulatory adaptor protein for FceprisonRI-mediated allergic reactions in both mast cells and basophils.

Tu101. Cross-Cohort Discovery and Validation of Two Conserved Asthma Endotypes Using Nasal Brushing Transcriptomics

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Abstract Text: Asthma remains clinically and biologically heterogeneous, limiting the consistency of treatment responses and motivating molecular stratification to guide precision therapy. In a recent real-world cohort of dupilumab-treated patients, 21.3% were non-responders and 47.1% were partial responders, underscoring unmet need for patient stratification (PMID: 38992817, 2024). To address this, we aggregated nasal brushing (NB) transcriptomic profiles from three adult asthma cohorts—CAAPA, ROCHE, and CAMP (PMIDs: 38806494, GSE41862, 29891868) —capturing approximately 400 unique patients. Using an established computational framework, we independently analyzed each cohort to discover conserved patient subpopulations, or disease endotypes based on molecular profiling data. Across studies, two highly reproducible endotypes emerged: Endotype I, enriched for immune cell activity and cytokine signaling; and Endotype II, enriched for ciliated/epithelial programs including cilium movement. Significant similarity in gene expression and pathway signatures was observed across cohorts, and permutation testing confirmed robustness ($p \ll 0.001$). Guided by these findings, we trained an NB-gene based endotype classifier in the training cohort (CAAPA), which spans 9% to 100% of African ancestry by genotyping, achieving 99% accuracy with Cohen's kappa = 0.97. Validation in independent cohorts maintained 81.5% accuracy (kappa = 0.63), demonstrating generalization beyond the training population. The resulting classifier is available as a research tool for post-hoc assessment of endotype–treatment response relationships in asthma clinical trials. In parallel, the conserved biology defining Endotype I and Endotype II provides a foundation for endotype-specific target discovery and hypothesis generation for precision therapeutics. Collectively, this work establishes a reproducible, NB-based molecular stratification for adult asthmatics and offers a practical path to connect disease biology with therapeutic benefit across diverse cohorts. By consolidating evidence across multiple independent datasets, leveraging an established endotyping framework, and validating performance in independent cohorts, we outline a scalable approach ready for exploratory analyses on additional immunological diseases. Future applications include powering post-hoc analyses of treatment response and enabling endotype-specific target discovery as additional datasets become available.

Tu102. Multi-mechanistic Approaches to Disarming IgE-mediated Allergic Disease

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Abstract Text: Objectives: Pathogenic IgE production drives a wide range of allergic and atopic diseases. When allergen-specific IgE binds to its high-affinity receptor FcεRI on basophils and mast cells, allergen-mediated cross-linking triggers the release of inflammatory mediators, causing allergic reactions. Seismic has developed multiple approaches to rapidly disarm allergic effector cells: (i) cleaving and eliminating circulating IgE, (ii) dissociating IgE from effector cells and down-modulating effector function, and (iii) sweeping IgE from circulation for accelerated IgE clearance. Here we focus on the discovery and design of our IgE dissociator, which rapidly dissociates IgE from FcεRI on effector cell surfaces, prevents effector cell activation, and blocks binding of free IgE to FcεRI and FcεRII. Methods: IgE dissociators, discovered through an immunization campaign, were screened by surface plasmon resonance and primary human cell-based assays using flow cytometry to assess their ability to dissociate IgE from FcεRI. Confirmatory basophil activation assays were performed on human whole blood. Pharmacokinetics (PK), pharmacodynamics (PD), and *in vivo* efficacy were tested in relevant preclinical models. Results: Seismic's proprietary IgE dissociator rapidly dissociates IgE from the high-affinity receptor FcεRI on basophil and mast cell surfaces without triggering unwanted degranulation. Like the standard of care (Omalizumab), our IgE dissociator

prevents free IgE from binding to FcεRI and FcεRII. However, unlike Omalizumab, it also acts on IgE already bound to FcεRI, leading to complete IgE dissociation and faster, more extensive FcεRI down-modulation than previously observed. Our IgE dissociator demonstrates favorable PK and PD, with superior efficacy in preventing antigen-induced systemic anaphylaxis. Conclusions: Seismic's IgE dissociator molecules address multiple aspects of IgE pathology by rapidly removing FcεRI-bound IgE and neutralizing free IgE without triggering effector cell activation. This provides a unique and potentially more effective approach to treating IgE-driven allergic and atopic diseases.

W100. CyToF Profiling Uncovers Disruptions in T Cell Regulatory Networks in Individuals Allergic to House Dust Mites

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Abstract Text: Allergic individuals who are immunologically sensitized to house dust mites (HDM), exhibit key features such as basophil activation and serological IgE positivity to Derp-1. Overall there is a failure to maintain tolerance to otherwise innocuous antigens, the disease mechanisms for which remains under investigation. This study aims to understand the regulatory deficits in HDM allergy with the aid of high parametric XT-CyToF analysis on a Singaporean cohort, where allergic prevalence of up to 70% has been reported. A population of allergic (n=23), atopic (n=16) and control individuals (n=16) were tested for serological IgE clinical reactivity to Derp-1 (ImmunoCap), with allergic individuals further reaffirmed with positive basophil activation against Derp-1. CyToF analysis of PBMCs revealed a distinct gross dysregulation in the CD4 Treg lineage. Agnostic FlowSOM cluster analysis of the Treg compartment, indicated a distinct segregation in the memory $CCR4^{+}CCR6^{+}$ Treg subsets, where allergic individuals ($p< 0.0001$) fail to activate (HLADR-), present an unstable ($CD154^{+}$) and dysfunctional phenotype (ICOS-Tigit-CD152-), which corresponds to a heighten level of both $CCR4^{+}CCR6^{+}$ B cells ($p< 0.01$) and $CD4^{+}IL\text{-}13^{+}IFNg^{-}$ T effector memory cells ($p < 0.0001$) in allergic individuals. Separately within the CD8 lineage, we discovered a similar regulatory deficit in B cell germinal centre targeting $CD8^{+}CD45RO^{+}CD27^{+}$CD154-PD1-$CXCR5^{+}$ (TfH) with TC1 regulatory capacity ($TNFa^{+}$, $IFNg^{+}$) in allergic individuals ($p< 0.0001$). In summary, we provide insights on regulatory deficits impacting HDM allergic individuals, that could reflect therapeutic potential.

W101. Dupilumab Treatment Outcomes in Pediatric Non-Esophageal Eosinophilic Gastrointestinal Disorders

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Abstract Text: Objectives: Eosinophilic gastrointestinal disorders (EGID) outside the esophagus are rare, Th2-mediated diseases with limited evidence-based therapies. We aimed to characterized outcomes in children with non-esophageal EGIDs treated with dupilumab. Methods: We reviewed pediatric patients with eosinophilic gastritis (EoG), duodenitis (EoD), and/or colitis (EoC) prescribed dupilumab at a single children's hospital from 3/2019-12/2025. Results: Twelve patients were identified. Median diagnosis age was 7.4 years (range 1.3-17.8). Ten (83.3%) were male, and all had overlapping EoE. Phenotypes included four EoG (n=4, 33.3%), EoD (n=1), $EoG^{+}EoD$ (n=6, 50%), and $EoG^{+}EoD^{+}EoC$ (n=1). Patients failed a median of four treatments (range 2-7), including proton pump inhibitors (91.7%), dietary elimination (83.3%), and swallowed topical (83.3%) or systemic corticosteroids (50%). Complications included poor weight gain (66.7%), steroid-induced adrenal insufficiency (58.3%), anemia (25%), and hypoalbuminemia (16.7%). Dupilumab prescribing indication and dosing was for EoE (83.3%), followed by asthma (8.3%) and eczema (8.3%). Eight patients (66.7%) demonstrated histologic remission

on dupilumab (median 8.0 months). Among five patients undergoing additional endoscopy on dupilumab, one achieved delayed histologic remission at 25.0 months, and four demonstrated sustained remission. At chart review, median dupilumab duration was 17.0 months (range 5.8-29.2). Eight patients (66.7%) demonstrated clinical response, nine (75%) remained on therapy, and three (25%) discontinued dupilumab due to peripheral eosinophilia (n=2) or lack of response (n=1). Dupilumab adverse effects were limited to mild injection reactions (n=3). Conclusions: Dupilumab can provide clinical and histologic improvements in pediatric patients with non-EoE EGIDs. Further studies should clarify treatment efficacy, ideal dosing and safety.

Autoimmune Diseases

Th102. CNP-104, an Antigen-specific Tolerogenic Nanoparticle Therapy, Stabilizes Prognostic Trajectory and Liver Stiffness with a Link to Reduction in Pathogenic T Cells in Primary Biliary Cholangitis

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Abstract Text: Background: Primary biliary cholangitis (PBC) is a progressive autoimmune liver disease with significant unmet need. Current standard-of-care treatments focus on bile acid regulation without directly addressing the underlying T Cell-mediated pathology. CNP-104 is a first-in-class tolerogenic nanoparticle encapsulating the dominant PBC autoantigen, PDC-E2, designed to restore antigen-specific immune tolerance. This analysis presents exploratory prognostic and liver stiffness data from a Phase 2a study of CNP-104. Methods: In this randomized, double-blind, placebo-controlled trial, 42 PBC patients with an incomplete response to ursodeoxycholic acid were enrolled. Participants were randomized 2:1 to receive two intravenous doses of CNP-104 (4 or 8 mg/kg) or placebo, one week apart. Exploratory endpoints included prognostic risk scores (UK-PBC, GLOBE, ALBI) and their components, liver stiffness measurement (LSM) via vibration-controlled transient elastography (VCTE, FibroScan®), and PDC-E2-specific Th17 T cells. Data from the go-forward 4 mg/kg CNP-104 dose ("CNP-104;" n=11) and placebo (n=16) cohorts are presented. Results: Through Day 270, UK-PBC, GLOBE, and ALBI prognostic risk scores worsened in the placebo arm but remained stable in the CNP-104 arm (p=0.010, p=0.013, p< 0.0001 vs. placebo, respectively), indicating a reduced risk of poor clinical outcomes. This impact on composite scores appears to be driven by stabilization of albumin in CNP-104 compared to worsening in placebo (CNP-104: +0.5±1.39g/L from baseline; placebo: –1.2±1.93g/L; p=0.014), suggesting an early positive effect on hepatic function. LSM, an independent predictor of clinical outcomes, increased in placebo but remained stable with CNP-104 through Day 365 (p=0.0013). Across a range of thresholds (%change from baseline), CNP-104 consistently yielded a higher proportion of subjects achieving stable/improved LSM compared with placebo (≥0% change at Day 120: 59% vs 18%; p=0.014). Mechanistically, patients who had a reduction in pathogenic PDC-E2-specific Th17 cells also had a significant decrease in LSM compared to those with an increase (p=0.001). Conclusions: CNP-104 treatment

stabilized both validated prognostic risk scores and liver stiffness, providing early evidence of disease-modifying activity. The correlation between modulated antigen-specific pathogenic T Cells and improved clinical endpoints suggests an effect mediated by restoring immune tolerance. These findings support the continued development of CNP-104 as a mechanistically distinct therapy for PBC in larger randomized trials.

Th103. Epstein-barr Virus Reprograms Autoreactive B Cells as Antigen-presenting Cells in Autoimmune Diseases

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Abstract Text: Introduction Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by anti-nuclear antibodies (ANA). Epstein-Barr virus (EBV) infection has been epidemiologically associated with SLE, yet its role in pathogenesis remains incompletely defined. Methods We developed an EBV-specific single-cell RNA-sequencing platform and used it to sequence EBV-infected B cells directlry isolated form the patient. Using combined EBV-seq, CITE-seq, BCR-seq, and scRNA-seq, we sequenced the EBV genes, B cell receptor (BCR) repertoires, and transcriptomes of blood B cells from patients with SLE . Results We demonstrated that, in SLE, EBV^+ B cells are predominantly $CD27^+CD21_{low}$ memory B cells that are present at increased frequencies and express CD70, ZEB2, TBX21, and antigen presenting cell transcriptional pathways. Integrative analysis of chromatin immunoprecipitation sequencing (ChIP-seq), assay for transposase-accessible chromatin (ATAC-seq), and RNA Polymerase II occupancy data revealed EBNA2 binding at the transcriptional start sites and regulatory regions of CD27, ZEB2, and TBX21 (Tbet), as well as the antigen presenting cell genes demonstrated to be up-regulated in SLE EBV^+ B cells. We expressed recombinant antibodies from SLE EBV^+ B cells and demonstrated that they bind prototypical SLE nuclear autoantigens, whereas those from healthy individuals do not. We further found that SLE EBV^+ B cells can serve as antigen presenting cells to activate T peripheral helper cells with concomitant activation of related EBV- anti-nuclear double negative 2 B cells and plasmablasts. Conclusion Our results provide a potential mechanistic basis for EBV being a driver of SLE through infecting and reprograming nuclear antigen reactive B cells to become activated antigen presenting cells with the potential to promote systemic disease-driving autoimmune responses.

Th104. Defining the Immunological Features of CD19 CAR T Cell Therapy in MRL/lpr Mice

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Abstract Text: Systemic lupus erythematosus (SLE) is a complex rheumatic disease that affects 3.4 million people worldwide and is characterized by a pronounced sex bias, with a female-to-male ratio of 9:1. CD19 chimeric antigen receptor (CAR) T cell therapy has been shown to be an effective treatment for SLE, leading to drug-free remission for at least two years. Despite this advance, questions remain about the immunological correlates of remission, often referred to as “immune reset,” and whether disease type or severity affect responses to or durability of this therapy. Here, we used MRL/lpr mice to comprehensively characterize the immunological outcomes of CD19 CAR T cell treatment and compared responses to MRL/lpr mice deficient in TLR9 and NOX2, both displaying exacerbated disease. First, we found that CD19 CAR transduction efficiency is significantly lower in cells from MRL/lpr mice compared to C57BL/6 mice and declines with age and symptom severity. Nonetheless, *in vivo* B cell depletion by CD19 CAR T cells was highly effective in all three strains of MRL/lpr mice. Notably, the wildtype MRL/lpr compared to $Tlr9^{-/-}$ and $Nox2^{-/-}$ MRL/lpr mice had a higher persistence of CAR T cells in the periphery, bone marrow, and spleen 18 weeks post-administration. We also observed that CD19 CAR T cell treatment reduced the frequency of

plasmacytoid dendritic cells, age-associated B cells, double negative T cells, and T regulatory cells. These effects were more pronounced in the Tlr9$^{-/-}$ MRL/lpr mice compared to the wildtype and Nox2$^{-/-}$ MRL/lpr mice, suggesting that CD19 CAR T cell therapy may be particularly effective in individuals with reduced TLR9 expression and consequent heightened TLR7 signaling, a key contributor to the sex bias observed in SLE. The frequencies of these pathogenic cell subsets also correlate strongly with B cell frequency in CD19 CAR T treated mice, irrespective of genotype, suggesting that sustained CAR-mediated B cell depletion underlies the observed improvement in disease outcomes. Overall, these results reveal broader immunological effects of CD19 CAR T cell therapy that contribute to our understanding of the immune reset phenotype and the durability of this treatment.

Th105. Not All Autoreactive B Cell Subsets Are Created Equal: Elucidating Their Unique Properties and Pathogenic Potential

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Abstract Text: INTRODUCTION: B cells play an essential role in T1D pathogenesis. They act as potent antigen presenting cells capable of priming autoreactive T cells; however, the mechanisms of pathologic B cell accumulation remain poorly understood. Once in circulation, autoreactive B cells are rendered anergic through mechanisms of peripheral tolerance. A population of anergic B cells have been described in a subpopulation of naïve IgM-/IgD^{+}/CD27^{-} B cells, coined Naïve IgD positive B cells (BND cells). We have identified a novel subset of BND cells, termed BND2 cells, that demonstrate an ability to escape anergy compared to traditional BND cells hereon referred to as BND1 cells. Importantly, BND2 cells are both expanded and enriched in autoreactivity in patients with T1D compared to controls. This project aims to identify mechanisms enabling BND2 cells to escape anergy, compared to traditional BND1 cells, potentially identifying an early lapse in immune regulation contributing the development of autoreactive T cells in T1D. METHODS: 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 to understand how differential interaction with self-antigen may contribute the breakdown of peripheral tolerance. Additionally, we leveraged cell culture and murine models of T1D to identify soluble factors contributing to BND2 cell development. RESULTS: 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. Additionally, we found that combined IFNγ and TLR7 stimulation is sufficient to convert BND1 cells into BND2 cells, independent of T cell help both *in vitro* and *in vivo*. CONCLUSIONS: Our findings demonstrate that BND2 cells escape peripheral tolerance through mechanisms involving IFNγ and TLR7 signaling. Additionally, we have identified distinctive physiochemical properties and V(D)J gene usage patterns in BND2 cells, highlighting a unique BCR repertoire that may contribute to this subset's ability to escape peripheral tolerance.

Th106. Impact of Competitive Interactions Between Bacteroides and Akkermansia on Type 1 Diabetes

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Abstract Text: Type 1 diabetes (T1D) is an autoimmune disorder characterized by T cell–mediated destruction of pancreatic β cells, leading to lifelong insulin dependence. While genetic susceptibility is a critical determinant of disease risk, accumulating evidence highlights the gut microbiota as a key environmental modulator of T1D pathogenesis. In particular, alterations in early-life microbial colonization, intestinal barrier integrity, and mucosal immune responses have emerged as interconnected factors influencing disease initiation and progression. While studies employing pre-clinical models have shown a disease preventive role for Akkermansia muciniphila, human gut commensals belonging to the Bacteroides genus, including Bacteroides fragilis and Bacteroides dorei, are increasingly recognized to have pathobiont effects in T1D. Our recent report using germ-free (GF) and specific

pathogen free (SPF) non-obese diabetic (NOD) mice has shown that gut colonization by B. fragilis results in suppression of mucin production, inhibition of colonization by specific microbial communities including A. muciniphila and acceleration of T1D progression. Hence, we hypothesized that Bacteroides members competitively inhibit T1D-protective commensals such as A. muciniphila from the colonic microbial niche and promote T1D progression. This hypothesis is being tested *in vitro* and by using GF and SPF NOD mice that are mono- and co-colonized using different human gut commensals. *In vitro* cultures demonstrate profound inhibitory effects of B. fragilis and B. dorei on A. muciniphila. Our co-colonization experiments show that both B. fragilis and B. dorei suppress gut colonization by A. muciniphila and promote a pro-inflammatory immune response and T1D disease progression. While A. muciniphila protects against insulitis, B. fragilis and B. dorei worsen it. Studies are currently underway using mouse models, organoids and epithelial cell cultures to determine the nature and dynamics of the competitive interactions between these human gut commensals and the impact on overall gut microbiota structure and function, intestinal and systemic immune responses, gut physiology, and T1D disease progression. These studies are expected to facilitate the development of microbial manipulation strategies for preventing T1D.

Th107. Longitudinal Single-cell Profiling Identifies Immune Signatures of TNF Inhibitor Response and Failure in Non-infectious Uveitis

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Abstract Text: Purpose: Although TNF inhibitors (TNFi) are widely used in the treatment of non-infectious uveitis (NIU), the mechanisms underlying treatment response remain unclear. The purpose of this study was to characterize immune cell alterations and molecular responses to TNFi therapy in non-infectious uveitis and investigate differences between clinical responders and non-responders prior to the initiation of therapy. Methods: Paired peripheral blood mononuclear cell samples (PBMCs) from 16 NIU patients were collected before starting TNFi treatment and while on TNFi therapy. Single-cell RNA sequencing (sc-seq), surface-protein (CITE-seq), and T Cell receptor (TCR-seq) sequencing were performed on PBMCs using the 10x Genomics platform. Data processing and analysis performed using Seurat v5.3. Results: At baseline, treatment responders showed higher expression of genes associated with immune activation and inflammatory signaling across lymphocyte subsets. Responders showed upregulation of MHC class II genes in activated $CD4^+$ and $CD8^+$ T cells together with elevated TNF pathway and effector genes including TNFAIP3, NFKBIZ, IFNG (activated $CD4^+$ T cells), and TNF and NFKBIZ (NK cells). Non-responders at baseline showed downregulation of TNF pathway genes, while B cells showed increased expression of stress associated genes (HSPA1A, LGALS1, and DDT), consistent with a cellular stress state. Samples obtained on treatment from clinical responders showed TNF/MHC related signatures (MHC class II genes in activated $CD8^+$ T cells and TNF in NK cells), which were already detectable in responders at baseline and remained present. In contrast, samples from treated non-responders showed broad upregulation of TNF-associated, stress response, and signaling regulatory genes across multiple immune cell types, most prominently in B cells and activated T cells. These included heat-shock and stress response genes. Overall, TCR analysis revealed decreased clonal expansion post-treatment. Conclusion: TNFi treatment response in NIU is characterized by suppression of inflammatory and stress response signatures across immune cell types whereas non-responders demonstrated persistent TNF driven activation and metabolic stress adaptation particularly within B cells and activated T cells.

Th108. Antibodies Against TRIM72 Protein Compromise Membrane Repair in Lupus Patient Derived Organoids

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Abstract Text: Background Defective plasma membrane repair has been implicated in heart failure, muscular dystrophy and neurodegeneration. We have previously shown that autoantibodies to membrane repair protein TRIM72 are found in patients with inflammatory myositis and that these antibodies negatively affect the membrane repair process. We report the presence of autoantibodies to TRIM72 in lupus patients with myocarditis and we describe the development of a novel, in-vitro, lupus patient iPSC derived cardiac organoid model which we utilize to examine the functional effects of these autoantibodies on membrane repair in human derived cardiac muscle cells. Methods A human iPSC line generated from a lupus patient was differentiated into cardiomyocytes (CMs), and cardiac organoids. Anti-TRIM72 antibodies in lupus patient serum were confirmed by ELISA prior to functional injury assays. To model membrane injury across tissues, we established complementary systems: (1) Laser-induced membrane injury in monolayer cardiomyocytes and 3D cardiac organoids, with membrane integrity quantified by FM4-64 dye uptake kinetics under defined conditions including lupus patient serum, healthy control serum, polyclonal anti-TRIM72 antibodies, normal IgG, and Ca^{2+}-free medium. (2) Mechanical injury of the sarcolemmal membrane of cardiomyocytes using glass-bead agitation, followed by lactate dehydrogenase (LDH) release quantification. Results Cardiac organoids formed hollow, spherical structures with spontaneous contractile activity and expressed cardiac muscle–specific markers, including TNNT2, as confirmed by confocal imaging. Lupus patient sera containing anti-TRIM72 autoantibodies or rabbit anti-TRIM72 antibodies markedly impaired both monolayer cardiomyocyte and 3D cardiac organoid membrane resealing, as compared to normal IgG and healthy serum controls. $Ca2^{+}$-free conditions further delayed repair, validating the assay's sensitivity to canonical membrane repair regulators. Consistent with these findings, mechanical injury of cardiomyocytes revealed significantly increased LDH release in the presence of lupus patient serum, suggesting that circulating antibodies to membrane repair can directly impair the physiologic intrinsic membrane repair in human cardiomyocytes and allow the release of intracellular components. Conclusion We developed a novel, patient derived testing platform that enabled mechanistic interrogation of membrane repair in a disease-relevant context. These data suggest that patient derived autoantibodies against TRIM72 play a significant role in lupus patient cardiac manifestations. Ongoing experiments will further define TRIM72-dependent mechanisms.

Th109. Elevated Influenza Antibodies in Recent Onset Type-1 Narcolepsy

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Abstract Text: Objective: Epidemiological studies have shown associations between pandemic H1N1 2009 Influenza-A infection and vaccination (only using Pandemrix®) and the onset of narcolepsy, an autoimmune disease strongly associated with HLA-DQB1*0602 and other immune genes such as, TRA and TRB. We tested whether recent onset type-1 narcolepsy patients have increased flu antibodies titers comparing with well-matched controls by serological experiments. Methods: Sera of 226 recent onset type-1 narcolepsy patients (24 [0.5-48] months) and 307 healthy controls matched by sex, age, and year and season of sample collection were used. Sera were tested for Influenza-A and B antibodies using hemagglutinin inhibition (HAI) assays and neuraminidase inhibition (NAI) assay against the dominant strains known to circulate at time of collection. 5 was assigned as antibody titer < 10. Geometric mean titers (GMT) were calculated by ten to the power of the average of log10 transformed antibody titers. Associations between confounding factors and disease status and HA/NA antibody titers were further analyzed using multiple variable linear and logistic regression. Results: Increasing GMT of HA antibody against H1N1pdm09 [OR=13.2(3.98, 43.7), p=0.00; β-coefficient=0.25(0.15,0.35), p=0.00] and B/Victoria [2.63 (1.06-6.46), p=0.04; β-coefficient=0.12(0.03, 0.22), p=0.01] was associated with onset of narcolepsy by multivariate logistic and

linear regression. For NA antibodies, elevated GMT of NA antibody against H1N1pdm09 [β-coefficient=0.31(0.15, 0.47), p=0.00], and B/Victoria [β-coefficient=0.19 (0.09-0.28), p=0.00] was found in patients by multivariate linear regression, whereas no association was found with HA and/or NA antibodies against H3N2 and B/Yamagata. HA and NA antibody titers against different strains on the same samples were weakly correlated (Pearson co-efficient [0.01 – 0.29]). Conclusion: Both H1N1pdm09 and B/Victoria, may trigger narcolepsy onset, but not other strains. This result is in line with a recent epidemiological study in Europe that reported a strong increase in narcolepsy onset in 2010 (following 2009 H1N1 pandemic) and a secondary peak in 2013 following a season with a dominant B/Victoria infection.

Th110. TNFR1 Deletion Protects Human Stem Cell–Derived Islets from TNF-Alpha Driven Inflammatory Injury

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Abstract Text: Background Stem cell–derived islet (SC-islet) transplantation offers a renewable source of insulin-producing cells for type 1 diabetes (T1D). However, inflammatory signals within the graft microenvironment promote β-cell dysfunction, death, and immune cell recruitment. Because many of these deleterious effects are mediated by TNF-α signaling through TNF receptor 1 (TNFR1), we hypothesized that selective deletion of TNFR1 in human SC-islets would confer cell-intrinsic resistance to TNF-driven injury. Objectives To generate and functionally characterize TNFR1-knockout ($TNFR1^{-/-}$) human SC-islets, evaluate their responses to TNF-α and pro-inflammatory cytokines *in vitro*, and establish assays to assess the impact of TNFR1 deletion in combination with HLA class I editing on resistance to T cell–mediated injury. Methods Human pluripotent stem cells were edited using CRISPR/Cas9 to delete TNFR1 and β2-microglobulin (B2M). Single- and double-knockout lines were differentiated into SC-islets alongside wild-type controls. β-cell differentiation and insulin/C-peptide expression were assessed by flow cytometry and reporter readouts. TNFR1 signaling was evaluated by measuring TNF-α–induced HLA class I upregulation. Inflammatory stress was modeled using TNF-α/IFN-γ/IL-1β, and β-cell death and insulin loss were quantified by apoptosis markers and reporter assays. T cell co-culture assays were established using PBMCs activated with anti-CD3/CD28/CD2 to assess direct and cytokine-mediated bystander injury. Results $TNFR1^{-/-}$ hPSC lines retained pluripotency and efficiently differentiated into C-peptide^{+} SC-islets. TNF-α–induced HLA class I upregulation was markedly reduced in $TNFR1^{-/-}$ SC-islets, confirming effective disruption of TNFR1 signaling. Exposure to TNF-α or cytokine cocktails induced β-cell death and insulin loss in wild-type SC-islets, both of which were significantly attenuated in $TNFR1^{-/-}$ SC-islets. In PBMC co-cultures, $B2M^{-/-}$ SC-islets were protected from direct cytotoxicity but remained susceptible to cytokine-mediated bystander killing. Preliminary data suggest that combined TNFR1/B2M deletion partially protects SC-islets from cell death under these inflammatory conditions. *In vivo* survival of $TNFR1^{-/-}$ SC-islets following transplantation into NSG mice is currently under investigation using bioluminescence imaging. Conclusions TNFR1 deletion preserves β-cell differentiation while conferring resistance to TNF-α–mediated injury. This platform enables mechanistic dissection of TNFR1-dependent inflammation and supports rational combination strategies with HLA class I editing to improve the durability of SC-islet replacement therapies for T1D.

Th111. High-dimensional Immune Profiling Identifies Chemokine-driven Tissue Trafficking in Active Systemic Sclerosis

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Abstract Text: Background Systemic sclerosis (SSc) is characterized by immune dysregulation, vasculopathy, and progressive fibrosis, yet the immunological mechanisms distinguishing active from inactive disease remain poorly defined. Clinical indices insufficiently capture dynamic immune processes that drive tissue inflammation and fibrosis. We hypothesized that active SSc is associated with coordinated peripheral immune signatures linked to chemokine-driven recruitment of pathogenic immune subsets into target tissues. Objectives To identify and characterize the composition and functional states of the peripheral blood-derived immune cells in patients with active SSc, inactive SSc and healthy controls. Methods Peripheral blood mononuclear cells from patients with active SSc (n=14), inactive SSc (n=26) and healthy controls (n=10) were profiled using high-dimensional mass cytometry (CyTOF) with disease specific antibody panel. Disease activity was defined using the EUSTAR activity index. Unsupervised clustering (FlowSOM), dimensionality reduction, supervised gating and immune network analyses were performed. Publicly available transcriptomic datasets from affected skin, lung and gastrointestinal tissues of SSc patients were re-analysed to interrogate the expression of chemokine ligands corresponding to receptors identified in peripheral immune subsets from CyTOF analyses. Results Active SSc demonstrated peripheral immune signatures distinct from inactive SSc and healthy controls. Naïve and transitional B-cell subsets with mucosal and tissue-homing receptors, including CCR6, were increased, suggesting enhanced migratory potential. The myeloid compartment was skewed toward $CD14^+$ monocytes with interferon-response signatures, implicating innate immune activation. NK cells showed a shift toward cytotoxic phenotypes. Circulating T Cell subsets expressing chemokine receptor combinations consistent with tissue egress were reduced, supporting active recruitment into inflamed organs. Innate-like MAIT cells expressing mucosal homing receptors and pro-fibrotic effector molecules were enriched in active SSc. Immune network analysis revealed increased network density and centralization in active disease, with a disease-specific module enriched for CLA^+ and $CCR4^+$ immune clusters, consistent with coordinated trafficking programs. Re-analysis of SSc tissue transcriptomes demonstrated upregulation of CXCL9, CXCL11, CXCL12, and CX3CL1, ligands for chemokine receptors expressed by peripheral immune subsets identified by CyTOF, providing mechanistic evidence of chemokine-mediated immune cell recruitment into affected tissues. Conclusion Active systemic sclerosis is characterized by coordinated peripheral immune dysregulation with chemokine-dependent trafficking linking peripheral immune states to tissue inflammation and fibrosis.

Th112. Therapeutic Dosing with Antigen-specific PLGA/CNP Nanoparticles Induces an Increase in Regulatory T Cells in the Spleen, Blood, and Disease-associate Tissue

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Abstract Text: Inflammatory immune cell infiltration, tissue destruction, and spread epitope-specific T cell activation underly $CD4^+$ T cell-mediated autoimmune disease pathogenesis. Investigational antigen (Ag)-containing biodegradable poly(lactide-co-glycolide) nanoparticle, i.e. tolerogenic immune-modifying particles/Cour Nanoparticles (TIMP/CNPs), treatment is both safe and efficacious for Ag-specific tolerance induction in mice and Phase I/IIa clinical trials in celiac disease primary biliary cholangitis, as well a in preclinical studies. Prior studies have shown a dependency of Ag-specific CNP treatment on treatment-induced regulatory T cells (Tregs) and Tr1 cells. The present studies utilized the transfer of congenically marked TCR transgenic T cells (5B6 T cells) into wildtype SJL mice to track these cells over time post treatment. Recipient mice were primed with PLP139-151/CFA and followed for disease. At the peak of acute disease, the mice were treated with either unloaded CNP or CNP-PLP139-151. At various time points post treatment, spleen, liver, CNS, and blood were collected to quantify and phenotype the 5B6 T cells. In continuing to identify the mechanism by which Ag-specific CNP treatment induces tolerance, the present data show that cDC2s are the APCs that present the CNP-associated Ag in a tolerogenic manner to Ag-specific $CD4^+$ T cells. Additionally, we identify that therapeutic treatment with Ag-specific CNP significantly decreases the number of effector $CD4^+$ T cells within the disease-associate tissue, while increasing the ratio of Ag-specific $CD4^+$ regulatory cells (Tregs) to IFN-gamma$^+$ cells. Consequently, treatment induced an increase in the number of Ag-specific Tregs, anergic $CD4^+$ T cells, and the ratio of Ag-specific Treg to IFN-gamma$^+$ cells

present within the spleen and blood. Additionally, we have identified that the percentage of both Ag-specific and host-derived Tregs increased within the blood post Ag-specific CNP dosing. Therefore, these data show that treatment-induced increases in Ag-specific $CD4^+$ T cells post dosing and cellular alterations across treatment relevant tissues.

Th113. Synovial NK Cells as Drivers of Inflammation in Juvenile Idiopathic Arthritis

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Abstract Text: Juvenile idiopathic arthritis (JIA) is a heterogeneous childhood-onset arthritis, with oligoarticular and polyarticular subtypes accounting for 60–80% of cases. The cellular mechanisms sustaining chronic synovial inflammation in JIA remain incompletely defined. Natural killer (NK) cells comprise 10–40% of lymphocytes in peripheral blood and synovial fluid; however, their functional contributions to JIA pathophysiology are not well characterized. We collected paired blood, synovial fluid, and synovial tissue samples from patients with JIA and profiled NK cell phenotype, function, and heterogeneity across these compartments. Synovial NK cells exhibited increased production of pro-inflammatory cytokines, including IFN-γ and GM-CSF, compared with circulating NK cells. Consistent with these profiles, NK cells enhanced activation of myeloid cells *in vitro*. Single-cell RNA sequencing demonstrated enrichment of activating receptor–associated transcriptional programs in synovial NK cells and identified synovial fibroblasts as a potential source of NK cell–activating signals. In a murine model of inflammatory arthritis, depletion of NK cells was associated with reduced disease severity. Collectively, these data support a model in which local stromal cues within the synovium modulate NK cell activation, promoting cytokine production and downstream myeloid cell responses that may contribute to synovial inflammation in JIA.

Th114. CD2-targeted Therapy in Type 1 Diabetes Promotes Treg and Exhausted T Cells

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Abstract Text: Objectives: The costimulatory molecule, CD2, is highly expressed on effector and central memory T cells relative to regulatory T cells (Treg) and may be an important target for immune modulation in type 1 diabetes (T1D). The phase 2 T1DAL trial (ITN045AI, NCT00965458), using a CD2-targeting fusion protein, alefacept, demonstrated preserved beta cell function with depletion of the effector T cell population and relative sparing of Treg. In a new clinical study, DESIGNATE (ITN095AI, NCT05574335), we evaluated whether CD2 T cell depletion with a monoclonal antibody, siplizumab (TCD-601, ITB-MED), resulted in a similar mechanistic outcome. Methods: The open-label DESIGNATE trial randomized T1D participants within 18 months of diagnosis to one of four siplizumab dosing arms, with 12 weekly doses administered. Using PBMC samples from baseline, during the treatment period, and up to 52 weeks from baseline, we characterized longitudinal changes in T cell and NK cell populations and quantified CD2 expression by flow and mass cytometry. Results: A total of seven participants were enrolled before the trial was halted for lymphopenia. Four participants discontinued treatment early due to lymphopenia, and three completed the 12-week course. The Treg:T effector ratio and the frequency of exhausted ($PD1^+TIGIT^+$) CD4 T cells increased in all participants. Participants who discontinued therapy saw greater increases in these values. While $CD2^{high}$ cells were depleted and $CD2_{low}$ cells spared in the completers, both $CD2^{high}$ and $CD2_{low}$ were depleted in the discontinuers. Discontinuers and completers had differing NK cell phenotypes at baseline, and discontinuers experienced a more rapid recovery of NK cells after depletion. Conclusions: In T1D

participants, a monoclonal antibody targeting CD2 increased relative levels of Treg and exhausted CD4 T cells but was associated with significant T cell depletion. These findings support the continued study of CD2-targeting molecules to develop new therapies for T1D

Th115. Defining the Differentiation Mechanisms of Pathogenic Granzyme K$^+$ CD8 T Cells

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Abstract Text: Objective: Our lab and others have identified a unique subset of granzyme K (GZMK)-expressing CD8 T cells that are enriched in tissues in rheumatoid arthritis (RA) and other diseases. These GZMK$^+$ CD8 T cells exhibit a transcriptional profile different from classic granzyme B (GZMB)-expressing cytotoxic T lymphocytes (CTLs). Instead, these CD8 T cells have low cytotoxic potential and express high levels of GZMK, a serine protease which activates complement cascades and stimulates stromal cells to produce pro-inflammatory cytokines. Despite these potent effector functions and observed abundance in multiple disease settings, the mechanisms driving the expansion and differentiation of GZMK$^+$ CD8 T cells remain unclear. In this study, we investigate the effects of high- and low-affinity T Cell receptor (TCR) stimulation as well as peptide stimulation on GZMK expression by CD8 T cells. Methods: We stimulated bulk CD8 T cells from healthy donors using anti-CD3 antibody clones OKT3 and UCHT1, which have high and low relative affinities for CD3 epsilon, or HLA-02*01-restricted immunodominant peptides from influenza, cytomegalovirus (CMV), and Epstein-Barr virus (EBV) for up to 4 days and measured GZMK and GZMB protein by flow cytometry and mRNA by RT-qPCR. Results: T cell stimulation using OKT3, a high-affinity anti-CD3 antibody clone, sharply reduced intracellular GZMK protein and increased intracellular GZMB protein. Lower-affinity anti-CD3 clone UCHT1 induced a milder reduction in GZMK at lower concentrations, suggesting that TCR stimulation regulates GZMK protein in an analog manner. Stimulation with influenza, CMV, or EBV peptides similarly induced a mostly GZMB$^+$GZMK- phenotype among tetramer-positive (i.e. antigen-specific) cells. Next, to determine whether observed GZMK protein reflects GZMK transcriptional expression versus release of GZMK protein with continued transcription, we performed RT-qPCR. Upon TCR stimulation, CD8 T cells rapidly produced high levels of GZMB transcripts but exhibited a sharp decrease in GZMK transcripts, indicating that TCR stimulation reduces GZMK expression at the transcriptional level. Conclusions: We demonstrate here a reductionist *in vitro* system to tease out the TCR-dependent cues involved in the differentiation of pathogenic GZMK$^+$ CD8 T cells. Ongoing studies will test the effect of co-stimulation, cytokine signaling, and repeated antigenic exposures on CD8 T cell phenotypes.

Th116. High Placental Transfer TNF Inhibitors During Pregnancy Are Associated with Delayed T Cell Maturation and Transient IL-12/IFN-γ Immune Pathway Dysfunction in Infants

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Abstract Text: Background: Tumor necrosis factor inhibitors (TNFi) used during pregnancy cross the placenta, particularly in the third trimester, and may persist in neonatal circulation for several months after birth. As TNF is essential for early lymphoid development and IL-12/IFN-γ–mediated responses to mycobacterial infection, prenatal TNFi exposure may disrupt neonatal immune maturation and increase the risk of immune dysfunction. Methods: We conducted a longitudinal evaluation of immune development in infants exposed to TNFi in utero, from birth (umbilical cord blood) through one year of age (peripheral blood). Analyses included: (1) lymphocyte subset development; (2)

cytokine responses to mycobacterial stimulation to assess IL-12/IFN-γ axis functionality; and (3) humoral immunity, including immunoglobulin levels and vaccine responses. Outcomes were compared with those from an age-matched healthy pediatric cohort. Clinical follow-up was performed by a pediatric immunologist, monitoring growth, infection incidence, and signs of immune dysregulation until one year of age. Results: Fifty infants born to mothers with rheumatic diseases or inflammatory bowel disease were enrolled, including pregnancies treated with TNFi (adalimumab [ADA], n=16; infliximab [IFX], n=11; etanercept [ETN], n=5; certolizumab pegol [CZP], n=11) and untreated controls (n=7). Infants exposed to TNFi with higher placental transfer (ADA and IFX) showed delayed T cell maturation during the first year of life, characterized by increased naïve T cells, reduced memory T cells, and decreased regulatory T cells at birth. In contrast, minimal immunological alterations were observed in CZP- and ETN-exposed infants, and B cell immunity remained largely intact across groups. Functional impairment of the IL-12/IFN-γ axis was detected at birth in ADA- and IFX-exposed newborns, with progressive recovery following postnatal drug clearance, supporting postponement of BCG vaccination until complete elimination of TNFi. Clinically, no increase in infection rates was observed; however, 12 of 50 children developed mild to moderate allergic manifestations, which were associated with reduced regulatory T cell frequencies. Conclusions: Exposure to TNFi during pregnancy may have lasting implications for neonatal immune development. Targeted immunological monitoring and specialized follow-up are warranted in exposed infants.

Th117. NanoGalaxy™, a Novel Hydrophilic Nanoparticle mRNA-based Platform, Induces T Cell Proliferation and Antigen-specific Immune Tolerance in Murine Autoimmune Disease Models

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Abstract Text: mRNA-based therapies are very promising in immunological diseases owing to their ability to encode virtually any antigen or immunomodulatory proteins. We utilized NanoGalaxy™, a hydrophilic nanoparticle (HNP) platform, to identify HNPs with mRNA delivery to spleen antigen-presenting cells (APCs) and optimal T cell priming to induce antigen-specific immune tolerance (ASIT) in autoimmune diseases. mRNA delivery to spleen APCs was assessed by intravenous (i.v.) injection of HNPs encapsulating GFP mRNA and flow cytometry in splenocytes. T cell activation was evaluated by i.v. injection of HNPs encapsulating ovalbumin (Ova) mRNA in mice and flow cytometry in splenocytes after immunizations. ASIT was assessed in two models, delayed-type hypersensitivity (DTH) induced with Ova protein immunization and experimental autoimmune encephalomyelitis (EAE) induced with myelin oligodendrocyte glycoprotein peptide (MOGp) immunization. For ASIT therapies, mice were injected i.v. with HNPs or lipoplex (LPX) encapsulating Ova or MOGp mRNAs plus/minus tolerogenic immune modulators. Therapeutic responses were assessed by measuring footpad inflammation (DTH) or clinical scores (EAE), and immune correlates were assessed by cytometric bead array (CBA) and/or flow cytometry in splenocytes. To assess translatability, Cynomolgus macaques received two i.v. doses of HNPs encapsulating Ova mRNA administered three weeks apart. T cell responses were assessed by FluoroSpot and safety was assessed by analyzing blood parameters. In mice, HNPs transfected over 15% APCs in spleen with minimal hepatic uptake, and several HNPs induced T cell responses that were higher compared to LPX benchmark. Of note, HNPs encapsulating Ova or MOGp mRNAs plus tolerogenic immune modulators efficiently induced ASIT in the DTH and EAE models, respectively, while HNPs without tolerogenic co-payloads did not lead to optimal immune tolerance. In Cynomolgus macaques, immunization with HNPs delivering Ova mRNA was well tolerated and induced antigen-specific T cell responses, without evidence of hepatic toxicity or changes in key blood parameters. In summary, NanoGalaxy™ HNP is a versatile platform to deliver mRNA-encoded antigens plus immune modulators to spleen APCs and induce ASIT in autoimmune diseases, while showing translational potential and safety in non-human primates.

Th118. Preclinical Development of an Anti-IL-22 Antibody for the Treatment of Atopic Dermatitis

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Abstract Text: The pathologic role of interleukin-22 (IL-22) in atopic dermatitis has been shown in a number of clinical settings, including improved AD symptoms in moderate to severe AD patients after anti-IL-22 and anti-IL-22RA1 inhibition and induction of AD in patients receiving exogenous IL-22 for treatment of non-dermatological disease. Where commercially successful anti-IL-4/13 receptor and anti-IL-13 antibody drugs inhibit the Th2 inflammatory processes inherent to AD, inhibition of IL-22 acts directly on the epithelium to address the dysregulated antimicrobial and barrier function of AD skin. Anti-IL-22 treatment represents both a compelling standalone therapy and a clear complement to existing drugs. To this end we developed a set of fully human anti-IL-22 antibody candidates using our proprietary Anthrobody® discovery platform and GLIMPSE antibody language model. Beginning with antibodies discovered directly from humans, we performed several rounds of engineering to optimize the stability, developability, and potency of the initial candidates. From these we have selected a lead candidate, IFX-101, which demonstrates high affinity (< 10pM) and *in vitro* potency (IC50 ≤ 10pM), as well as strong *in vivo* potency evidenced by significant reduction in ear thickening even at low (0.1mg/kg) dosing. In addition to superior potency, IFX-101 is half-life extended to a projected human half-life of ~76 days, which enables a patient preferred quarterly dosing profile or better. We believe that the combination of a distinct mechanism of action relative to existing therapeutics and superior drug exposure and dosing schedule enabled by the long half life of this candidate will have a positive impact on AD patients, particularly those for whom existing therapies have failed.

Th119. Characterization of B Cells in Dermatomyositis and Sjögren's Syndrome

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Abstract Text: Introduction and aim: Dermatomyositis (DM) is a rare, heterogeneous and often severe autoimmune disease characterized by skin and/or muscle inflammation, which is linked to various autoantibodies (1). Sjögren's syndrome (SS) is a disease characterized by autoimmune exocrine gland inflammation and destruction, associated to varied systemic features such as joint, skin or neurologic symptoms (2). Recent data show a role for B cells in these diseases, and B cell targeted therapies have demonstrated promising results (1)(3). However, the knowledge about B cell contribution is still limited, while morbidity and mortality remain high. Hence, this study aims at better understanding the role of B cells in the pathogenesis of DM and SS, to improve treatment strategies. Methods and design: We included 7 DM patients (2 anti-Jo1, 1 anti-PL7, 1 anti-TIF1γ, 1 anti-MDA5, 2 anti-NXP2), 4 SS and 5 healthy controls (HC). Whole B cells were analyzed using 10X single cell RNA sequencing including whole transcriptome and VDJ sequencing. Results: We included a total of 106'151 B cells, with 51'100 DM cells, 36'918 SS cells and 18'133 HC cells. After dimension reduction and unsupervised clustering from a principal component analysis-reduced gene expression matrix, we defined 9 clusters. 6 included CD27- cells, and 3 included $CD27^+$ cells. One of the CD27- clusters showed a gene expression pattern suggestive of an atypical/age associated B cell-like subset ($CD19^+$, $CD20^+$, $CD21^-$, $Zeb2^+$, $Tbet^+$, $CD11c^+$). In DM and SS patients compared to HC, we observed shifts in the number of cells in several CD27- cell types, including in an $HLA\text{-}DR^+CD74^+$ cluster in DM ($p = 0.016$) and in a $PTEN^+$, $PAX5^+$ cluster in SS ($p = 0.020$). CDR3 Shannon diversity index shows a significantly higher diversity in most DM and SS CD27- subsets compared to HC. Conclusion: These data are in line with literature showing distorted B cell characteristics in autoimmune diseases, in particular naïve population shifts and BCR changes, and may be linked to tolerance checkpoint defects (4). Uncovering the role of B cells in DM and SS may open new avenues for understanding the pathogenesis of these conditions and may promote new therapeutic approaches.

Th120. Elevated Baseline CD4⁺ T Cell Activation State Is Associated with Poorer Response to Abatacept in Type 1 Diabetes

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Abstract Text: Abatacept is a CTLA-4 Ig fusion molecule that blocks CD28-mediated T cell activation. Trials of abatacept in patients with type 1 diabetes (T1D) have yielded mixed clinical outcomes and inconsistent correlations between immune parameters and clinical response. Thus, the DREAMT (NCT04118153) mechanistic clinical trial enrolled individuals recently diagnosed with T1D, treated weekly with subcutaneous abatacept, and conducted intensive immune profiling and a 6-month quantitative response (QR) measure integrating age and C-peptide to compare observed versus expected (with and without abatacept) disease progression. DREAMT participants displayed a mean positive QR ($p < 0.05$), indicating three months of abatacept significantly delayed T1D progression. Longitudinal PBMC samples were characterized by flow and mass cytometry and immune changes from baseline were evaluated using the Wilcoxon signed-rank test. Abatacept reduced the expression of costimulation markers ICOS, CTLA-4, and OX40 to varying degrees in memory CD4⁺ T regulatory (Treg) and T conventional (Tconv) cells. As early as two weeks after treatment, surface and intracellular ICOS and intracellular CTLA-4 declined in both populations while surface CTLA-4 remained comparable to baseline throughout treatment ($p < 0.05$), suggesting that abatacept limits CTLA-4 recycling. Intracellular and surface OX40 were reduced in memory Treg at two and four weeks, respectively, and remained relatively stable in Tconv ($p < 0.05$). In addition, total %Treg, %ICOS⁺PD1⁺ of T follicular helper (Tfh) and Treg cells, and %CCR7-PD1⁺ of Tfh and T peripheral helper (Tph) cells declined within two weeks of treatment ($p < 0.05$), consistent with observations at later timepoints in previously published trials. However, these early treatment-induced changes in CD4⁺ activation did not correlate with clinical response. Instead, QR was negatively associated with baseline CD4⁺ T cell activation, including increased frequencies of ICOS⁺PD1⁺ and CTLA-4⁺ memory Treg ($r = -0.59, -0.52$), as well as OX40 expression and IL-2 production by Tfh ($r = -0.49, -0.46$). Higher baseline %TNFa production by memory Tconv and PD-L1 expression across antigen presenting cell subsets were also associated with poorer response (Spearman, $p < 0.05$). Together, these findings suggest that while abatacept broadly reduces CD4⁺ T cell activation, elevated baseline CD4⁺ immune activation predicts poorer clinical response.

Th121. Parathyroid Hormone–B Cell Axis in Rheumatoid Arthritis: Defining PTHR1 as a Potential Biomarker of Disease Activity

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Abstract Text: Introduction: Hormonal–immune crosstalk is increasingly recognized in autoimmune pathogenesis. Beyond estrogen and prolactin, parathyroid hormone (PTH) is emerging as an immune regulator through its receptor PTHR1, expressed on lymphocytes. We have previously reported that PTHR1 expression is significantly elevated in B cells from patients with systemic lupus erythematosus. Building on these findings, we examined whether similar changes occur in rheumatoid arthritis (RA) and their association with disease activity. Methods: Peripheral blood samples from patients with RA (n = 25) and age-matched controls without autoimmune disease (n = 11) were analyzed by flow cytometry to quantify %PTHR1⁺ B cells and mean fluorescence intensity (MFI). Group comparisons used Mann–Whitney U tests, and correlations with CDAI, CRP, ESR, PTH, and vitamin D employed Spearman coefficients. Results: RA patients exhibited higher %PTHR1⁺ B cells. PTHR1 MFI was significant elevated in patients vs. controls ($p = 0.047$). Within RA, PTH correlated positively with %PTHR1⁺ B cells ($r = 0.31$, 95% CI −0.13–0.65) and CDAI ($r = 0.25$, 95% CI −0.18–0.59), though not reaching significance. Vitamin D levels were lower in RA ($p = 0.013$), and inverse trends with disease activity were observed. Interestingly, of the 25 patients with RA, 17 had elevated PTHR1 expression on B cells and decreased vitamin D levels. Conclusions: We demonstrate that PTHR1 expression is upregulated in B lymphocytes from RA patients, supporting the existence of

a PTH–PTHR1–vitamin D axis that influences B-cell activation and inflammation. These findings position PTHR1 as a promising biomarker and mechanistic link between endocrine and immune dysregulation in RA.

Th122. Characterization of Intestinal Bacterium Specific T Helper Subsets in Individuals with Rheumatoid Arthritis

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Abstract Text: The mucosal origins hypothesis proposes that immune dysregulation in individuals who develop rheumatoid arthritis (RA) is initiated at mucosal surfaces, promoting subsequent autoimmunity and joint pathology. Evidence supporting this model includes the emergence of IgA anti-citrullinated protein antibodies (ACPA), which precede clinical disease by several years and correlate with mucosal inflammation. The intestinal bacterium Subdoligranulum didolesgii (S. dido) was identified as a target of RA-associated IgA and IgG autoantibodies. Gavage with S. dido induced $CD4^+$ T cell activation, autoantibody production, and joint swelling in murine models, establishing a causal relationship between this gut microbe and RA-relevant immune responses. However, T cell responses against S. dido in human subjects with RA remain incompletely characterized. To elucidate precise antigenic targets of S. dido–specific T cell responses, unbiased HLA class II peptidomic profiling of HLA-DRB1*04:01 (DR0401) transgenic dendritic cells pulsed with killed S. dido was performed, resulting in the identification of DR0401-bound peptides from multiple S. dido proteins. After synthesizing the most prevalently observed peptides, *in vitro* T cell assays were conducted to screen for S. dido–derived epitopes that elicited measurable $CD4^+$ T cell responses. After confirming the resulting epitopes through T cell cloning and additional testing, a multicolor HLA class II tetramer panel incorporating the most prevalent S. dido epitopes was developed. This panel was applied for direct *ex vivo* profiling of S. dido–specific $CD4^+$ T cells in the peripheral blood of RA patients and matched controls, providing insight into disease-associated effector T cell states, including inflammatory and memory subsets implicated in RA pathogenesis. In summary, this study verifies a human-relevant antigenic and immunologic framework by which an intestinal microbe may initiate RA-associated autoimmunity. Identification of S. dido-derived HLA-restricted epitopes and characterization of cognate T cell responses establishes a foundation for antigen-specific immune monitoring and informs future strategies for early disease interception, mucosal immune modulation, and restoration of tolerance prior to irreversible joint damage.

Th123. Nasal Foralumab Ameliorates Neuroinflammation in Secondary Progressive Multiple Sclerosis by Inducing Transcriptional Reprogramming in T Cells

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Abstract Text: Chronic neuroinflammation in multiple sclerosis (MS) can persist independently of relapse activity, revealing mechanisms of progression not addressed by current therapies. Dysregulated T Cell activity plays a central role in this process and is the main target of nasal Foralumab immunotherapy. Here, we aimed to identify CNS-related biomarkers in the CSF of MS patients linked to disease progression and to define the molecular effects of Foralumab in secondary progressive (SP) MS. We integrated longitudinal single-cell transcriptomic data from PBMC (n = 4) with data-independent acquisition (DIA) proteomics from CSF (n = 22) collected at baseline, 3 months, and 6 months from multiple sclerosis patients treated with nasal foralumab. Differential gene expression analyses comparing 3- and 6-month time points with baseline revealed downregulation of inflammatory response pathways, interferon signaling, and NF-κB activity in T cells. Co-expression network analysis demonstrated a shift in naïve $CD4^+$ T cells toward a regulatory-permissive transcriptional state, while Tregs exhibited a distinct transcriptional program enriched for tissue-migration markers. Peripheral effector $CD8^+$ T cells showed reduced cytotoxicity-

associated co-expression modules. In CSF proteomics, SP samples were enriched for fibroblast-related proteins involved in tissue injury, fibrosis, and extracellular matrix (ECM) remodeling (FAP, COL8A1), along with elevated cytotoxicity markers (CTSW) and components of the complement cascade. Foralumab treatment significantly reduced the expression of proteins linked to cytotoxic activity (LAMP1), interferon signaling (IFNAR1), tissue injury/fibrosis (COBA1), and NF-κB–dependent inflammation (SIGLEC14, LY86). Notably, increased TGFB1 expression was observed in both CSF and PBMCs, suggesting a regulatory and immunomodulatory effect of the therapy. We identified accessible biomarkers distinguishing SP patients and proteins linked to disease progression and found that nasal Foralumab ameliorates MS by increasing TGFB1 and suppressing T cell cytotoxicity and inflammation.

Th124. Potent and Selective First-in-class Oral IRF5 Degrader, KT-579, Inhibits Endosomal TLR-induced Responses in SLE Derived PBMCs and Significantly Reduces Disease Activity in the MRL.lpr Mouse Lupus Model

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Abstract Text: IRF5 is a transcription factor and master regulator of pro-inflammatory immune responses activated downstream of specific pattern recognition receptors (PRRs) like Toll-like receptors (TLRs). IRF5 is constitutively expressed in immune cells such as monocytes, dendritic cells and B cells. Human genetic and functional studies have linked IRF5 dysregulation to the pathogenesis of multiple autoimmune diseases, including SLE and Sjögren's, and Irf5-deficient mice are protected from lupus onset and severity. In SLE, endosomal TLRs recognize nuclear self-antigens, and can trigger IRF5 activation to drive the breakdown of immune tolerance via a cascade involving increased Type I IFN and pro-inflammatory cytokine production, and cellular functions such B cell activation and autoantibody production. Despite its strong mechanistic and genetic validation, IRF5 has historically remained undrugged, likely due to its lack of catalytic activity, activation complexity and multiple functional isoforms making it well suited for targeted protein degradation. KT-579, an oral IRF5 degrader, has demonstrated potent and selective activity in preclinical studies, offering a novel approach to modulating this context-dependent central activator of immune responses. *In vitro*, peripheral blood mononuclear cells (PBMCs) derived from healthy or SLE donors with or without common polymorphisms associated with SLE were cultured with KT-579 in the presence or absence of TLR7, TLR8 or TLR9 activation to evaluate KT-579 potency and functional activity. KT-579 demonstrated potent IRF5 degradation equally across healthy and SLE donors and blocked TLR-induced nuclear IRF5 translocation which correlated with reduction of pro-inflammatory cytokines and IgG levels. *In vivo* KT-579 led to dose-dependent IRF5 degradation and inhibition of both TLR7- and TLR9- induced plasma cytokine release including TNFα, IL-6, IL-12 and IFNβ. In the MRL.lpr lupus model, KT-579 doses achieving >85% degradation led to significant reduction of proteinuria and 100% survival through end of study, reduction of both anti-dsDNA and anti-Sm autoantibodies in plasma, and reduction of IgG deposition in kidney, with results superior to active comparators including a TLR7/8 small molecule inhibitor. These findings position KT-579 as a novel, first-in-class, oral IRF degrader with the potential to transform the treatment landscape of lupus and multiple autoimmune diseases driven by IRF5 dysregulation.

Th126. An *in vivo* CRISPR Screen to Identify Genes Important for Patrolling Monocytes in Lupus

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Abstract Text: Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease. Patrolling or non-classical monocytes (pMonos), defined as CD14dimCD16hi cells in humans and Ly6CloTREML4^{+} cells in mice, are found in increased numbers in blood, skin and kidneys of SLE patients and are associated with lupus nephritis, a cause of morbidity and mortality in individuals with SLE. However, we have an incomplete understanding of the differentiation and function of pMonos during homeostasis and disease. To better understand pMono differentiation from classical monocytes (cMonos) and identify new therapeutic targets for SLE, we performed an *in vivo* CRISPR knock-out screen using a TLR7-overexpressing mouse model of SLE. We targeted 73 monocyte-expressed genes whose expression correlates with SLE genome-wide association study (GWAS) risk alleles according to eQTL studies. We infected Cas9-expressing hematopoietic stem cells with guide RNA (gRNA) libraries targeting these genes, as well as positive and negative control gRNAs, and transferred them into lethally irradiated mice. After 2 weeks, we sequenced gRNAs from spleen monocyte populations to identify genes that impact monocyte prevalence. We discovered genes that disproportionately reduced pMono prevalence compared to that of classical monocytes, including Ikzf1, Mapk14, and Ube2h. We focused particularly on Ikzf1, which encodes the transcription factor Ikaros, and which we have confirmed as a regulatory of pMono differentiation using single gRNA experiments *in vivo*. Using single cell RNA-seq of monocyte populations, we find that the Ikzf1 program is induced by TLR7 and is highly active in pMono vs. cMono. Collectively, these data define Ikaros as a critical regulator of pMono differentiation. Therapeutics targeting human IKZF1 are currently in clinical trials for SLE, and our work contributes contextual clues for its efficacy.

Th127. Efficacy of Teplizumab in Delaying Onset of Stage 3 Type 1 Diabetes

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Abstract Text: Background: Teplizumab is the first FDA approved medication to delay stage 3 Type 1 Diabetes (T1D) onset when used to treat stage 2 T1D. Teplizumab is an immunomodulator that attenuates autoimmune pancreatic beta cell destruction, although its clinical efficacy remains underreported. Methods: We report a single-center experience of teplizumab infusions for stage 2 T1D. Patients with stage 2 T1D were treated with teplizumab according to the label instructions. Diabetes medical history and adverse events were recorded prior to and at regular intervals after teplizumab. Progression to stage 3 T1D was defined as insulin initiation or meeting ADA diagnostic criteria. Results: As of December 1, 2025, our observational study included 15 enrolled patients who received teplizumab, 14 of whom completed the 14-day course. All treated patients had stage 2 T1D, identified through screening due to T1D family history (67%) or a new prediabetes diagnosis (33%). Three patients, all of whom completed the full teplizumab course, progressed to stage 3, between 36 and 233 days following treatment. At 6 months after teplizumab, 86.7% had not reached stage 3 (95% CI, 0.71-1.00). At 1 year after treatment, 79.4% had not reached stage 3 (95% CI, 0.612-1.00). With no additional stage 3 diagnoses, 79.4% of patients remained in stage 2 for up to 2 years post treatment. Serious adverse events during treatment were limited to two patients with cytokine release syndrome, both treated with steroids. One of these patients had a concomitant liver injury leading to treatment cessation after 5 doses of teplizumab, which resolved with a steroid taper by 12 days after teplizumab cessation. Neither effected patient reached stage 3 after 20 months of surveillance. In the month following treatment, two adult patients developed EBV reactivation, one adult patient had strep throat, and one pediatric patient was hospitalized for serum sickness, which was treated with prednisone. Conclusion: In a single-center experience, progression to stage 3 T1D following standard of care teplizumab was higher than rates following teplizumab in the pivotal clinical trial, but lower than rates in the trial's placebo group. Patients should be followed after the infusion to monitor for adverse events.

Th128. A Tissue-restricted Transgenic NOD Model to Study APC-specific Antigen-specific Tolerance *in vivo*

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Abstract Text: Re-establishing antigen-specific tolerance is a major goal in Type 1 diabetes (T1D) therapy to prevent β cell loss. Antigen-specific approaches improve on conventional immunotherapies by targeting autoreactive lymphocytes through controlled autoantigen exposure. These strategies employ diverse delivery modalities and target various antigen-presenting cells (APCs) to induce tolerance, yet optimal tolerogenic conditions remain undefined. We generated a novel mouse strain, termed "Inducible Diabetogenic Endogenous AntigenS" (IDEAS), carrying a Cre-inducible transgene encoding multiple T1D-relevant β cell epitopes and GFP on the non-obese diabetic (NOD) background. Low-level engagement of a few adoptively transferred islet antigen-reactive (IAR) $CD4^+$ and $CD8^+$ T cell clones revealed slight transgene leakiness and antigen presentation in IDEAS lymph nodes and spleen, whereas GFP was induced only upon Cre expression. We used this model to assess the tolerogenic potential of fibroblastic reticular cells (FRCs) using Ccl19CrexIDEAS mice. More pronounced activation of IAR T cell clones was observed after transfer into these mice, in which full expression was restricted to $Ccl19^+$ FRCs. Both IDEAS and Ccl19CrexIDEAS mice were protected from diabetes, and most IDEAS mice remained protected after anti-PD1 treatment. Lymph nodes from Ccl19CrexIDEAS donors were transplanted into immunodeficient NOD.scid recipients. Three weeks later, transferred IAR T cell clones could migrate to the grafted lymph nodes, where they were engaged by antigen-expressing FRCs. Ongoing incidence studies are evaluating the outcomes of this engagement on T cell phenotype and disease progression in NOD mice. In parallel, a non-leaky Cre-inducible version of IDEAS is being developed.

Th129. Adoptive Transfer of Lupus Patient PBMCs Promotes Salt-sensitive Hypertension and Kidney Injury in Immunodeficient Mice

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Abstract Text: Introduction: Salt sensitivity of blood pressure (SSBP) is an independent risk factor for cardiovascular disease (CVD), even in normotensive people. Systemic lupus erythematosus (SLE) is also a risk factor for CVD and hypertension. SLE and CVD are on the rise, and it is unknown if this is attributable to the increased dietary salt in the modern diet. Hypothesis: We hypothesized that the adoptive transfer of peripheral blood mononuclear cells (PBMCs) from patients with SLE/lupus into immunodeficient mice (NSG/MHCI/$MHCII^{-/-}$) would increase inflammation and SSBP after high salt feeding. Method: *In vitro* studies were performed using PBMCs isolated from patients with SLE and treated with and without high salt. We humanized mice with PBMCs from patients with SLE (n=7) and controls without autoimmune disease (n=7) in animal studies. The mice were fed a 4% high-salt diet for two weeks. Blood pressure was measured with radiotelemetry, immune phenotyping was performed with flow cytometry, and vascular reactivity was assessed in mesenteric arteries using wire myography. Results: A high salt diet significantly increased systolic blood pressure in lupus mice compared to the controls (133.0 vs.116.4 mmHg, p=0.001). APC infiltration (macrophages, monocytes, dendritic cells), proinflammatory markers, and oxidative stress (Isolevuglandins, Nox2) were significantly higher in the kidneys of mice receiving the PBMC from the SLE patients (lupus mice). Innate and adaptive immune cells and proinflammatory markers were significantly elevated in the kidney, spleen, and aorta. These mice also exhibited mesenteric artery vascular dysfunction ($p< 0.05$). Lupus mice excreted more urinary albumin than the controls (25.89 vs. 17.24 μg/ml p=0.142), indicating kidney injury. High salt treatment *in vitro* increased intermediate and nonclassical monocytes with increased CD86, IsoLG, and TNF-alpha expression compared to normal salt-treated cells. 3D mitochondrial analysis revealed increased mitochondrial fragmentation in high salt-treated monocytes, indicating salt-induced morphological

alterations. In conclusion, our findings suggest that immune cells from SLE patients, when exposed to high salt, promote salt-sensitive hypertension (SSBP) and a proinflammatory state in the kidney, aorta, and spleen. These changes impair vascular function, alter mitochondrial morphology, and exacerbate albuminuria in immunodeficient mice.

Th130. Dysregulation of the Extrafollicular B–T Cell Axis in Childhood-onset Systemic Lupus Erythematosus

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Abstract Text: Systemic lupus erythematosus (SLE) is a clinically heterogeneous autoimmune disease characterized by autoantibody production against nuclear antigens. Childhood-onset SLE (cSLE) represents 20% of all SLE cases and is associated with a more severe disease course underscoring the need to dissect cSLE pathogenesis. SLE patients have increased frequencies of polyreactive B cells and expanded peripheral helper (Tph) and follicular helper (Tfh) CD4 T cell populations relative to healthy controls (HC). Under physiological conditions, polyreactive B cells produce broadly neutralizing antibodies that control viral infections such as flu, Epstein-Barr virus and SARS-CoV-2. However, the mechanisms of how polyreactive B cells are dysregulated in cSLE and their interactions with CD4 T cells remain poorly understood. We hypothesize that upon viral infection B cells in both HC and cSLE patients produce polyreactive antibodies that in cSLE patients become autoreactive. We further posit that these B cells activate their cognate CD4 T cells, thereby propagating autoimmunity. To address this hypothesis, we constructed barcoded nuclear-antigen specific B cell tetramers to sort antigen specific B cells alongside Tph and Tfh cells from cSLE patients and HC. We performed single-cell multi-omics on these sorted populations to understand transcriptomic, proteomic and repertoire differences. We observed clonal expansions of autoantigen specific B cells accompanied by clonal expansions within Tph and Tfh populations. Nuclear antigen specific and polyreactive B cells from cSLE patients exhibited a pro-inflammatory transcriptional program with upregulation of antigen presentation pathways. These cells also showed increased expression of activation markers compared with polyreactive B cells from HC, indicating a breach of anergy in polyreactive B cells in cSLE. In future work, we will address viral reactivity of these B cells by cloning their B cell receptors both in their germline and hypermutated forms and testing the resulting monoclonal antibodies against autoantigen and viral antigen arrays. Additionally, we will express the T Cell receptor sequences of the clonally expanded Tfh and Tph clones and assess their specificity for nuclear peptides using T cell hybridomas. Together, these studies will shed light on the role of polyreactive B cells, their viral reactivity and activation of cognate CD4 T cells in cSLE.

Th131. BTK Inhibition Resets Pathological B and T Cell Subsets in Patients with IgG4-related Disease

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Abstract Text: IgG4-related disease (IgG4-RD) is a fibroinflammatory disorder marked by tissue infiltration of $IgG4^+$ plasmablasts (PB) and elevated serum IgG4 levels. Bruton's Tyrosine Kinase (BTK) inhibition blocks key signaling pathways in B cells and showed promising efficacy in other autoimmune diseases. This is the first trial testing BTKi in IgG4-RD, and its effects were analyzed on a single-cell level. 10 patients with IgG4-RD affecting the submandibular and/or lacrimal glands were enrolled in this single-site, open-label, investigator-initiated trial (NCT04602598). Patients received zanubrutinib orally (80mg BID) for up to 24 weeks. The primary endpoint was the change in glandular volume at week 24 compared to baseline. Single-cell transcriptomes and immune repertoires of PBMCs were analyzed at baseline, week 12, and week 24 and compared with matched healthy controls. Two patients terminated at week 4 due to adverse events. For the 8 remaining patients, treatment with zanubrutinib 80 mg PO BID resulted in significant reductions in lacrimal (–45.4% change) and submandibular gland volumes (–30.0% change, both $p < 0.001$) at week 24. Serum IgG4 levels decreased by a mean of 413 mg/dL and the IgG4-RD

Responder Index decreased by a mean of 4.6 points (p = 0.02). Single-cell sequencing analyses of peripheral blood B cells found increased B cell receptor (BCR) activation and antigen presentation in IgG4-RD patients, and a significant subset of elevated genes in these pathways were downregulated by zanubrutinib. Zanubrutinib also normalized $IgG4^+$ PB and atypical B cell (ABC) counts that were elevated in IgG4-RD patients at baseline. Strikingly, zanubrutinib most strongly affected $IgG4^+$ and $IgG2^+$ PB, restoring the compositions of Ig subclasses to those seen in healthy donors. Numbers and activation levels of proliferating $CD4^+$ T cells correlated with $IgG4^+$ PB and ABCs, supporting the hypothesis that an extensive B cell / T cell cross-talk drives IgG4-RD pathology. In summary, we show that zanubrutinib inhibits pathogenic B and T cell subsets in IgG4-RD in a targeted fashion and is a promising therapeutic for IgG4-RD.

Th132. Subjective Fatigue Assessed by FACIT-F and BFI Questionnaires Correlates with Objective Wearable-derived Data in Patients with Rheumatoid Arthritis

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Abstract Text: 【Background/Purpose】Fatigue profoundly impacts quality of life in rheumatoid arthritis (RA), yet assessment relies on subjective patient-reported outcome (PRO) measures (PROMs) such as functional assessment of chronic illness therapy-fatigue (FACIT-F) and brief fatigue inventory (BFI). These questionnaires are prone to recall bias and fail to capture real-time fluctuations. This study aimed to clarify the association between wearable-derived data and fatigue severity via correlation analysis and machine learning.【Methods】This prospective observational study enrolled patients with RA who continuously wore a Fitbit. FACIT-F/BFI scores were correlated with 7-day preceding wearable data (< 80% wear time excluded), adjusting for covariates. Binary classification models were constructed using wearable data to predict severe (lowest quartile) vs. non-severe (highest quartile) PRO status, excluding intermediate quartiles. Five-cross validation and SHAP analysis were performed to identify key features and assess model robustness.【Results】Our study enrolled 107 patients with RA. FACIT-F scores indicated that No patients reported no fatigue (FACIT-F>40), and 1.9% had mild fatigue (FACIT-F 31–40). Moderate fatigue (FACIT-F 21–30) was observed in 19.6% of patients, while severe fatigue (FACIT-F≤21) was present in 80.4%. BFI scores showed that 26.4% of patients reported no fatigue (BFI=0), 54.7% had mild fatigue (BFI 1–3), 17.9% had moderate fatigue (BFI 4–6), and only 1.0% had severe fatigue (BFI 7–10). Correlations with an absolute correlation coefficient $|r| \geq 0.4$ are shown below. FACIT-F scores were correlated with resting heart rate ($r=-0.40$) and nocturnal HRV ($r=-0.40$). BFI scores were correlated with daytime HRV ($r=0.42$) and nocturnal HRV ($r=0.43$). FACIT-F binary classification model showed performance: accuracy 0.774, precision 0.703, recall 0.789, F1-score 0.742, ROC-AUC 0.88. BFI binary classification model showed performance: accuracy 0.773, precision 0.668, recall 0.828, F1-score 0.735, ROC-AUC 0.82.【Conclusion】A correlation was observed between self-reported fatigue and heart rate variability (HRV) and activity metrics from wearable data. The binary classification model achieved excellent performance, demonstrating that wearable devices serve as superior real-time fatigue biomarkers. M HK and KI are contributed equally.

Th134. Nerve Injury-induced Protein-1 (Ninj1) Deficiency Aggravates Murine Lupus Through Increased Neutrophil Extracellular Trap (NET) Formation

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Abstract Text: Background/Purpose: Nerve injury-induced protein-1 (Ninj1) is an adhesion molecule that plays various roles in immune and stromal cells, including the modulation of inflammation and a critical role during cell death. Dysregulation of inflammatory pathways and cell death has been linked to the development of autoimmune diseases, such as systemic lupus erythematosus (SLE). Therefore, we hypothesized that Ninj1 may be involved in the pathogenesis of SLE. Methods: Ninj1-deficient ($Ninj1^{+/-}$) and wild-type (WT) C57/B6 mice were induced to develop lupus-like autoimmunity using two induced models triggered by topical application of the Toll-like receptor 7/8 (TLR7/8) agonist imiquimod (IMQ) or by intraperitoneal injection of pristane. We assessed the mice for clinical phenotype, immune dysregulation, and organ damage. Results: In comparison to WT mice, $Ninj1^{+/-}$ mice exposed to IMQ exhibited greater splenomegaly at the expense of extramedullary hematopoiesis, more severe anemia, decreased spleen lymphocyte counts, and increased spontaneous neutrophil extracellular trap (NET). While there were no differences in autoantibody levels or proteinuria, $Ninj1^{+/-}$ mice developed more lupus-associated renal disease, as evidenced by increased immune complex deposition and exacerbated kidney inflammation and damage. In the pristane model, Ninj1 deficiency worsened diffuse alveolar hemorrhage and lung inflammation. Further analysis confirmed increased serum levels of genomic and mitochondrial circulating DNA in $Ninj1^{+/-}$ mice compared to WT mice, along with enhanced expression of proinflammatory and type I interferon (IFN)-related genes in the affected tissues. Conclusion: Preliminary, these findings suggest that Ninj1 deficiency aggravates murine lupus, at least in part, by increasing NET formation and circulating DNA. We are currently evaluating the effects of Ninj1 deficiency on specific molecular pathways in affected tissues to understand their implications for autoimmune diseases.

Th135. Deep Profiling of Lupus Nephritis Kidneys Reveals Dynamic Changes in Immune Cells Associated with Disease Progression

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Abstract Text: Lupus nephritis (LN) is a common, potentially fatal manifestation of systemic lupus erythematosus. Gaining mechanistic insights into the immune responses underlying LN and their relation to the histologic heterogeneity observed in this disease is essential for the development of improved therapies. To this end, single-cell RNA-sequencing (scRNA-seq) was used to profile dissociated kidney samples from 156 LN patients and 30 healthy individuals. Spatial transcriptomics (ST), utilizing a customized gene panel, was applied to kidney samples acquired from 6 LN patients and 2 healthy controls. Our analyses indicated that an increase in irreversible tissue damage, as measured by the NIH chronicity index (CI), is associated with a gradual switch of the local immune response from one dominated by monocytes, macrophages and cytotoxic cells and centered in and around the glomeruli, to one affecting the tubulointerstitium (TI) and featuring expanded $CD4^+$ T, $GZMK^+CD8^+$ T, B and dendritic cells (DCs), with a parallel decrease in the interferon response. In proliferative/mixed LN only, the degree of active inflammation correlates with expansion of disease-associated macrophage (DMac) subsets, which later contract as the CI increases. Trajectory analysis of the scRNA-seq data suggested that DMacs arise from both infiltrating monocytes and tissue-resident macrophages; this was supported by the ST data, as well as cell culture experiments. We provide evidence that DMacs interact with parietal epithelial cells, promoting the development of glomerulosclerosis. Characterization of inflammatory aggregates in the TI identified a number of aggregate subtypes, including one dominated by CCR7 DCs, Tregs and stem-like CD4 T cells; interestingly, effector CD4 T cells in this subtype demonstrated increased activation markers, suggesting that the Tregs were ineffective in

controlling these cells. Another subtype featured increased levels of highly activated B cells and pDCs, and was associated with elevated markers of tubule cell injury and a local increase in the interferon response. A third aggregate subtype was marked by a specific subset of DMacs and myofibroblasts, consistent with the role of the former cells in promoting fibrosis. Our work provides a detailed picture of the changes in the kidney immune mechanisms in LN as this disease progresses.

Th136. Accelerated Identification and Validation of Therapeutic Targets for Systemic Lupus Erythematosus

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Abstract Text: AbbVie's Immunology Discovery Research aims to enhance the clinical success rate of novel therapeutic targets by integrating disease-associated translational and functional datasets. This includes multi-OMICS data from patient samples, an internal semi-supervised machine learning model for target prioritization, and CRISPR-based approach for functional validation. This combined approach is particularly relevant for autoimmune disorders with heterogeneous clinical presentation and poorly understood underlying mechanisms of disease, including systemic lupus erythematosus (SLE). Here we performed comprehensive molecular profiling using PBMCs from a cohort of 144 SLE patients and 40 matched healthy controls (HC), with equal representation from Caucasian and African American donors. PBMCs were profiled using several techniques, including CITEseq. This approach enabled the identification of distinct molecular signatures in SLE samples compared to HC, as well as the validation of robust targets detected across multiple data layers. We observed a strong interferon signature and expansion of pathogenic lymphocyte subsets, particularly in patients with moderate and severe disease. Our analysis also pinpointed key molecular targets linked to disease severity. To establish mechanistic linkage between translational data and disease, we developed a multi-parametric high-throughput CRISPR knock out platform to evaluate target genes in relevant functional assays using human primary immune cells, such as B cells, dendritic cells, and T cells—key players in SLE pathophysiology. From approximately 100 prioritized targets, several emerged as the top candidates in regulating both B cell and dendritic cell functions, thus creating an opportunity for curative therapies simultaneously targeting multiple disease mechanisms. These findings deliver novel insights into the gene regulation of SLE in pathogenic immune cell types, deepen our understanding of SLE pathogenesis and reveal promising therapeutic targets for its treatment. Abbvie Disclosure statement: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Th137. First Oral Small-molecule Inhibitors Designed by Generative AI Achieve Biologics-like Pan-inhibition of IL-17AA, AF, and FF Dimers

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Abstract Text: The most effective therapies for immunological diseases are injectable biologics that many patients cannot access and often prefer to avoid. There remains a strong need for safe and effective oral treatment options. Targeting clinically validated cytokine pathways with small molecule modulators provides a viable route to first-in-class oral medicines. IL-17A and IL-17F are key drivers of multiple autoimmune diseases, yet small-molecule inhibitors developed to date have been restricted to IL-17A and lack activity on IL-17F. Building on AI-generated scaffolds, we developed orally bioavailable small-molecule IL-17 inhibitors that potently block the full spectrum of IL-17A- and F-containing dimers. Candidate inhibitors were engineered via AI-enabled structure-based generative design and virtual screening. Our lead inhibitors demonstrate high-affinity binding to all relevant isoforms and disrupt

receptor engagement. They achieve Ki < 0.1 nM for IL-17A and < 10 nM for IL-17F in cellular assays. The activity on IL-17A is over 10-fold more potent than leading IL-17A-specific small molecules. These inhibitors demonstrate favorable oral bioavailability and dose-dependently suppress IL-17F–driven cytokine production *in vivo*, achieving maximal inhibition levels comparable to the dual-specific antibody bimekizumab. These data present the first orally bioavailable small molecules with strong IL-17F engagement, exceptional potency against all IL-17A and F dimers, and robust *in vivo* activity. By combining the efficacy of dual-cytokine blockade with the convenience of oral dosing, these inhibitors represent a potential best-in-class opportunity for next-generation autoimmune therapies.

Tu103. Beyond Inflammation：anti-fibrotic Efficacy of TL1a Antibodies in a TNBS-induced Chronic Colitis Model in Humanized Mice

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Abstract Text: Background Inflammatory bowel disease (IBD) is characterized as a chronic and recurrent autoimmune disorder affecting the intestinal tract. While considerable emphasis has been placed on anti-inflammatory properties in current industrial research for novel IBD drug development, less attention has been directed toward anti-fibrotic effects. To establish a model that better recapitulates human IBD, we established a TNBS-induced chronic colitis model, characterized by sustained inflammation, progressive fibrosis, and immune dysregulation. Methods TL1A-IL23A-IL12B humanized mice were treated with TNBS once per week for six weeks. Disease Activity Index (DAI) score was used to estimate the severity of colitis. In addition, colon tissues were also subjected to histological and fibrosis evaluation. For biochemistry analysis, we quantified the levels of different cytokines and chemokines, as well as collagen deposition in the colon tissue. Furthermore, a multi-omics approach was conducted to delineate underlying mechanisms and responses of key signaling pathways involved in the development of fibrosis. Results Mice in the Vehicle group exhibited reduced body weight and DAI score was significantly elevated, confirming successful establishment of the model. Promisingly, TL1A antibodies administration significantly improved these two factors of treated mice. Furthermore, pathological and IHC staining revealed attenuated intestinal fibrosis, as evidenced by reduced expression of markers related to fibrosis. Correspondingly, significantly reduced TGF-β1 levels and diminished activation of fibrotic signaling pathways in the colon tissue were observed. Conclusions TNBS-induced chronic colitis model was successfully established. The animals manifested typical clinical symptoms such as diarrhea, hematochezia, serve body weight loss, along with increased colon density, elevated pathological scores, and upregulated expression of fibrosis markers. Treatment of Risankizumab and/or TL1A inhibitor markedly ameliorated the severity of colitis, as evidenced by improved clinical parameters (reduced weight loss and better stool consistency), lower colon density, and ameliorated pathological outcomes, alongside reduced fibrosis markers, mediated via the IL23/p19 and TL1A pathways. Our newly established model specifically targets intestinal fibrosis, a critically overlooked aspect of IBD pathogenesis. By shifting the focus beyond mere anti-inflammation, our team provides a superior experimental platform for developing novel therapeutical strategies, aiming at the current unmet need for effective anti-fibrotic treatments.

Tu104. Efficacy and Mechanisms of TCE-mediated B Cell Depletion in Humanized Systemic Lupus Erythematosus Model

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Abstract Text: Background: Systemic lupus erythematosus (SLE) is a clinically heterogeneous autoimmune disease with persistent therapeutic challenges. Although current therapies primarily target B cells, their clinical translation remains limited by species-specific differences in murine models. Fully humanized SLE models provide a bridge for preclinical evaluation of emerging therapies such as T cell engagers (TCEs). However, their mechanisms of action in SLE are not fully understood. This study aims to investigate the efficacy and underlying mechanisms of B-cell-targeting TCE in a humanized SLE model and inform preclinical drug development. Methods: A pristane-induced SLE model was established in HSC-NOG-EXL humanized mice via a single intraperitoneal injection of pristane. After 8 weeks of SLE development, TCEs were administered twice weekly for 3–4 weeks, with endpoint analyses performed at 10–11 weeks post-injection. Plasma levels of anti-dsDNA/histone antibodies, total IgG, and IgM were measured by ELISA. Immune cell subsets in the spleen and peripheral blood were analyzed by flow cytometry. Renal IgG deposition was evaluated via immunohistochemical staining, and cytokine profiles were quantified using Olink assay. Results: The pristane-induced humanized SLE model generally exhibited a common set of features that mirror the clinical features of human SLE. Total IgG, IgM, and autoantibodies were representative indicators for SLE diagnosis. The deposition of human IgG in the kidneys was also critical for SLE progression. Compared to normal mice, the pristane-induced groups showed increased levels of autoantibodies, IgG, and IgM. TCEs attenuated the pristane-induced lupus-like symptoms, including reductions in the levels of autoantibodies, IgM, and IgG. Reductions of B and T cell subsets in the peripheral blood and spleen suggested that the use of TCEs inhibited the number and function of B cells. Conclusion: This study conducted a detailed comparative analysis at the cellular and protein levels in a fully humanized SLE model. The model treated with B cell-targeting TCEs showed significant effects on B cell depletion, reduction in the levels of autoantibodies, and decreased deposition of human IgG in the kidneys. The study demonstrated that the fully humanized SLE model is a powerful tool for evaluating large molecule biologics, providing a robust platform for preclinical drug development.

Tu105. Rapid Immune Modulation in Systemic Lupus Erythematosus Through T Cell–Redirected Depletion of CD19^{+} B Cells After Single Target Dose

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Abstract Text: Dysregulated B-cell responses play a central role in the pathogenesis of systemic lupus erythematosus (SLE), contributing to autoantibody production, antigen presentation, and amplification of inflammatory immune circuits. Redirecting cytotoxic T cells to eliminate pathogenic B cells may enable more rapid and profound immune modulation. YK012 is a CD19×CD3 bispecific T Cell engager designed to induce targeted T Cell–mediated depletion of CD19-expressing B cells. Here we report the safety, pharmacokinetic (PK), pharmacodynamic (PD), and preliminary clinical activity from the dose-escalation stage of an ongoing Phase Ib study of YK012 in patients with moderate-to-severe SLE. Methods In this open-label Phase Ib/II study, adult patients with active SLE receiving stable background therapy received single intravenous target doses of YK012 at 1000 μg, 2000 μg, or 3000 μg (n=3 per cohort). The primary objective was safety and tolerability. Secondary and exploratory endpoints included PK, peripheral B-cell dynamics, serologic biomarkers, and disease activity assessed by SLEDAI-2K. Results Nine patients completed the dose-escalation stage. YK012 was generally well tolerated, with no dose-limiting toxicities or treatment-related serious adverse events. Cytokine release syndrome occurred in 8 patients (88.9%) and was all limited to grade 1–2 events; no grade ≥3 CRS or neurotoxicity was observed. Rapid systemic exposure was observed following infusion (median Tmax ~2–3 hours), with terminal half-life ranging from approximately 38 to 84 hours. Robust pharmacodynamic activity was observed across cohorts. Peripheral CD19^{+} B

cells declined rapidly following treatment, with reductions of approximately 80–96% from baseline and ≥90% depletion achieved in multiple subjects within the first month. These changes were accompanied by improvements in serologic disease markers, including decreases in anti-dsDNA antibodies and increases in complement levels (C3/C4) among patients with abnormal baseline values. Strikingly, early clinical improvements were observed, including rapid reductions in SLEDAI-2K scores within weeks after just one target dose of YK012 treatment. Conclusions Targeted T Cell–mediated B-cell depletion with YK012 demonstrated a manageable safety profile and rapid immunologic activity in patients with SLE. The combination of controlled CRS, profound B-cell depletion, and early improvements in serologic and clinical disease measures supports further evaluation of YK012 as a potential immune-resetting therapy for broad autoimmune diseases.

Tu106. Donor-derived CD8$^+$CD122$^+$ Tregs Generated in Mixed Donor Chimeric NOD Mice Delete Autoreactive T Cells

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Abstract Text: The establishment of mixed hematopoietic chimerism is a promising way to induce immune tolerance for islet replacement therapy and to treat the underlying autoimmunity in Type 1 diabetes (T1D). Mixed chimerism not only promotes effective thymic negative selection of autoreactive cells but also restores regulatory T cell (Treg) function and peripheral tolerance. In the current study, we determined that a novel class of donor-derived CD8$^+$CD44$^+$CD122$^+$ Tregs (d-CD8$^+$CD122$^+$ Tregs) plays a crucial role in controlling autoimmunity in non-obese diabetic (NOD) mice with induced mixed chimerism. Using adoptive T cell transfer experiments, we showed that d-CD8$^+$CD122$^+$ Tregs abrogate autoimmunity by selectively depleting the exogenously injected diabetogenic T cells in Recombination-Activating Gene deficient NOD mice. These d-CD8$^+$CD122$^+$ Tregs from NOD chimeras show upregulation of Helios, Programmed cell death protein 1, perforin, granzyme-B, CD39, Folate receptor 4, and downregulation of proinflammatory markers like Scart1 and Scart2. Using *in vitro* assays, we show that d-CD8$^+$CD122$^+$ Tregs respond specifically to a Complementarity-Determining Region-3 peptide sequence derived from T cell receptors of islet antigen-specific autoreactive T cells. Thus, mixed chimerism might be a method to revitalize CD8$^+$CD122$^+$ Tregs which are decreased in number and functionality in NOD mice. Similarly, we found that individuals with T1D have a deficiency in CD8$^+$CD122$^+$ Tregs, suggesting a potential loss of regulatory function accompanies disease onset. Revitalizing CD8$^+$CD122$^+$ Tregs may offer a new therapeutic strategy of restoring immune tolerance in autoimmune diabetes.

Tu107. MicroRNA-155 Regulates Neuroinflammation-mediated Gut Barrier Dysregulation and Dysbiosis

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Abstract Text: Multiple sclerosis (MS) is a chronic autoimmune and neurodegenerative disease caused by the host's immune cells attacking the myelin sheath in the central nervous system (CNS). While gut microbes play significant roles in regulating experimental neuroinflammation, MS and other neuroinflammatory diseases are also linked to substantial alterations in gut microbial taxa and intestinal barrier dysfunction. However, the molecular mechanisms that regulate neuroinflammation-induced dysbiosis remain to be determined. MicroRNAs (miRNAs), small, noncoding RNA molecules, regulate immune cell activity and differentiation. In experimental autoimmune encephalomyelitis (EAE), a model of MS, miRNA-155 is overexpressed. Elevated miRNA-155 levels have been shown to regulate T cell differentiation into pro-inflammatory phenotypes. Additionally, miRNA-155 may regulate the

intestinal epithelial barrier. We hypothesized that increased miRNA-155 expression during EAE could disrupt the intestinal microenvironment. We examined how miRNA-155 deficiency affects the MOG35-55 model of EAE in male and female C57BL/6 mice, along with its impact on EAE-induced intestinal barrier disruption and microbial dysbiosis. We first observed that EAE promotes sex-dependent early effects on colon transcriptomics and microbiota profiles in mice before the onset of EAE. Furthermore, we observed that miRNA-155 levels were higher in EAE mice than in healthy controls. miRNA-155 deficiency triggered sex-dependent effects on EAE. Moreover, miRNA-155 deficiency reversed the intestinal barrier disruption induced by EAE. Gut microbiota analysis showed that miRNA-155 deficiency led to notable changes in gut bacterial composition compared with wild-type controls, with these differences becoming more pronounced after EAE induction. Furthermore, miRNA-155 directly altered the composition of bacteria isolated from the gut of healthy mice. In addition, a novel anti-inflammatory mechanism to deliver miRNA-155 antagomir is proposed as a potential therapeutic strategy against miRNA-155-dependent autoimmune and inflammatory diseases. Our findings suggest that miRNA-155 plays a key role in regulating intestinal barrier function and the microbiota, contributing to the intestinal dysregulation observed in neuroinflammatory diseases.

Tu108. A New Mouse Model for Studying AQP4 Autoimmunity and Neuromyelitis Optica Spectrum Disorder

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Abstract Text: Background: Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune astrocytopathy driven by pathogenic autoantibodies targeting the water channel aquaporin-4 (AQP4) on the astrocytic endfeet. There remains a need for *in vivo* models that reliably develop both autoimmunity and pathogenesis in the same animal for preclinical NMOSD research. Objective: To develop and test a novel transgenic mouse strain in which AQP4 is constitutively absent but can be selectively re-expressed in astrocytes using Cre-mediated recombination. This design enables controlled temporal restoration of AQP4 after the immune system has encountered antigen. Methods: A loxP flanked LacZ sequence was inserted in exon 0 of the Aqp4 gene (AQP4.LacZ) thus removing the start codon. AQP4 expression was examined by quantitative reverse transcriptase, immunoblotting and immunohistochemistry in various tissues and primary astrocyte cultures from AQP4.LacZ mice. AQP4 function was assessed by detecting intracellular calcein fluorescence quenching as a measure of cell volume change in response to hypotonic challenge in cultured astrocytes. To develop an animal model of NMOSD, anti-AQP4 immunity was induced in the AQP4.LacZ mice with AQP4 peptide 135-153, then AQP4 expression was restored in astrocytes by intravenous administration of adeno-associated virus (AAV)-mediated Cre recombinase transduction under GFAP (glial fibrillary acidic protein) promoter.Clinical symptoms were evaluated by scoring animals' motor abilities and immunohistochemistry was used to check for neuroinflammation. Results: molecular techniques confirmed the loss of Aqp4 transcript and protein in the AQP4.LacZ mouse. Subsequent Cre induction under GFAP promoter restored AQP4 expression in AQP4.LacZ animals. The time to peak swelling and regulatory volume decrease were significantly blunted in response to hypotonic challenge in primary cultured astrocytes from AQP4.LacZ mice compared to wild type, while AAV-Cre recombinase partially restored AQP4 expression and function. Following re-expression, mice that were injected with AQP4 peptide 135-153 developed clinical and histopathological features of NMOSD, including motor weakness, and immune cell infiltration of the spinal cord. Conclusion: The AQP4.LacZ mouse is a AQP4 null strain that allows for temporal and cell-specific controlled AQP4 re-expression. This model offers a valuable system for NMOSD mechanistic and preclinical therapeutic studies by providing a physiologically relevant, flexible, and translationally meaningful tool.

Tu109. Quantitative Spatial Mapping of Macrophages in the Human Pancreas Across Stages of Type 1 Diabetes

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Abstract Text: Objectives Macrophages are contributors to type 1 diabetes (T1D), but their spatial distribution and activation state in human pancreas across disease stages remain incompletely defined. Our objective was to evaluate the extend and characteristics of macrophage infiltration in non-diabetic, autoantibody-positive (Aab^+), and T1D pancreata. Methods FFPE pancreatic sections of non-diabetic, T1D and Aab^+ donors were stained by conventional immunofluorescence for insulin, CD68, HLA class II, and Hoechst (nuclei).Whole-slide images were acquired using Zeiss AxioScan.Z1 widefield slide scanner, and high-resolution images of islets using Zeiss LSM880 confocal microscope.Whole-slide image datasets were subsequently processed and quantitatively analyzed in QuPath using a semi-automated machine-learning workflow, including region-based analyses (islet, peri-islet, exocrine), and expanded concentric ring analyses around islets.Data from more than 24 million cells was retrieved and spatially analyzed.Posteriorly, additional pancreatic sections were stained with a highly multiplexed 17-marker immunofluorescence panel and scanned at high resolution using the Orion platform (RareCyte).Semi-automated image analysis is currently ongoing using supervised and unsupervised machine learning algorithms in those whole-tissue images. Results Macrophage infiltration was significantly increased in T1D pancreata compared with Aab^+ and non-diabetic donors ($CD68^+$ cells: 8.6±4.7% vs 3.2±2.7% vs 1.5±0.5%; p=0.01). Infiltration in T1D appears contingent on the presence of insulin-containing islets. Large case-by-case variation was observed within the Aab^+ group, which some donors exhibiting higher rates of macrophage infiltration above the non-diabetic levels. Macrophages were most enriched in peri-islet regions independently of disease status, and expanded ring analyses showed a marked increase within 25 μm of the islet border, the closer ring to the islet. Macrophages with high HLA-II expression were strongly enriched in islet and peri-islet regions in T1D, and the proportion of $CD68^+HLA\text{-}II^+$ cells near islets strongly correlated with stressed pancreatic beta cells that also expressed HLA-II ($r=0.74$, $p< 0.0001$). The extended analysis of macrophage functional subsets defined by multiple phenotypic markers is work in progress. Conclusions These findings support a role for macrophages in human T1D initiation and progression. This work also highlights the value of semi-automated quantitative whole-slide image analyses. High-quality multiplex data with subcellular definition will provide further insights into the activation state of macrophages across disease stages.

Tu110. mRNA LNP Encoded Autoantigens Induce Durable Antigen-specific Regulatory T Cell Responses and Therapeutic Protection in Autoimmunity

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Abstract Text: Antigen-specific immune tolerance remains a central challenge in autoimmune disease, where current therapies rely on broad immunosuppression rather than selective immune reprogramming. We developed an mRNA lipid nanoparticle (mRNA LNP) platform designed to deliver disease-relevant antigens *in vivo* to promote antigen-specific regulatory T cell (Treg) responses. Using myelin oligodendrocyte glycoprotein (MOG) as a model $CD4^+$ T cell autoantigen, intravenous administration of MOG-encoding mRNA-LNP in mice induced robust expansion of antigen-specific $CD4^+$ T cells with dominant $FOXP3^+$ Treg skewing across dose levels. Similar antigen-specific $CD4^+$ T cell expansion with preferential Treg differentiation was observed in non-human primates following repeat dosing. In a MOG/CFA-induced experimental autoimmune encephalomyelitis (EAE) model, mRNA-LNP treatment prevented disease onset in a prophylactic setting and stabilized disease when administered therapeutically at initial clinical signs. Protection was durable, extending beyond 30 days prior to disease induction, consistent with establishment of antigen-specific T cell memory. Mechanistic analyses demonstrated that tolerance

was dictated by the encoded antigen rather than being an intrinsic property of the LNP formulation. Self-antigen MOG preferentially promoted regulatory differentiation, whereas foreign epitopes drove effector responses. Moreover, sequence-defined TCR contact residues influenced $CD4^+$ T cell fate outcomes, indicating that antigen sequence directly shapes immune programming. Together, these findings establish mRNA-LNP delivery of autoantigens as a programmable platform for durable antigen-specific tolerance. Future integration of immunopeptidomics-guided epitope discovery and fit-for-purpose antigen engineering or co-formulation with an immunomodulator may further enable precision antigen-specific immunotherapy tailored to disease biology.

Tu111. Enabling Diagnosis, Monitoring, and Precision Medicine in Lupus: A Strategic Roadmap for Biomarker Translation

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Abstract Text: Objectives The objective of this work was to develop a strategic roadmap for accelerating the clinical translation of lupus biomarkers. The effort began as a Lupus Research Alliance (LRA) initiative to guide strategic biomarker programs but grew into a broader set of recommendations for the field. Methods Surveys of patients, clinicians, and researchers were performed to identify and prioritize clinical unmet needs for biomarkers. A landscape analysis of the lupus biomarker literature was conducted to characterize the current research state and highlight exploratory candidates. Recommendations for targeted research and strategies to overcome translational barriers were then developed through expert surveys and structured discussions with a committee of researchers, clinicians, and industry specialists. Results The roadmap identifies and targets three priority clinical needs: (1) clearer diagnosis of systemic lupus erythematosus (SLE) and neuropsychiatric SLE (NPSLE), (2) sensitive monitoring of disease activity, and (3) predictive/prognostic markers to guide lupus therapy. It outlines an assessment of the current state of the field within each need area and provides recommendations to propel clinically useful biomarker innovation. Highlighted recommendations include developing “staging” biomarkers and AI-powered tools for marker and symptom pattern recognition to shorten diagnostic delays. The roadmap also recommends strategies such as the use of advanced imaging techniques to augment diagnostic biomarker discovery in NPSLE. Other recommendations include suggestions for developing more sensitive activity biomarkers through longitudinal validation and translation of relatively mature urine-based lupus nephritis biomarkers. The roadmap also spotlights the urgent opportunity for connecting biomarkers of heterogeneity to actionable clinical interventions. Uniting all the focused recommendations is a broader proposal to establish translational consortia-based efforts that provide a vehicle for bridging discovery and validation, enable sample and data sharing, and elucidate regulatory and reimbursement paths. Conclusions The roadmap charts a focused path for aligning discovery with prioritized contexts of use, investing in analytical and clinical validation, and mobilizing flexible, pre-competitive consortia that engage regulators, payors, industry, and patients. The LRA is well-positioned to bring these groups together and unite the lupus community toward improving outcomes with biomarkers. Finally, our approach can serve as a blueprint for similar assessments in other disease areas.

Tu112. Synthetic Progestins and Natural Progesterone Differentially Regulate Human Innate and Adaptive Immune Responses

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Abstract Text: Progestins, synthetic derivatives of progesterone used in oral contraceptives, are among the most widely used hormonal compounds globally, yet their immunological consequences remain poorly understood. Epidemiological studies have linked oral contraceptive use to altered risk of autoimmune diseases including multiple sclerosis, lupus, and rheumatoid arthritis, suggesting that synthetic progestins, which differ substantially in structure from natural progesterone, may dysregulate immune homeostasis in ways that are clinically consequential. Therefore, we investigated how natural progesterone (P4) and commonly used synthetic progestins differentially shape innate and adaptive immune responses in primary human cells. Peripheral blood mononuclear cells (PBMCs) from 39 healthy female donors were exposed to physiological concentrations of P4 and representative synthetic progestins across multiple generations. Cytokine profiling showed that levonorgestrel and drospirenone induced markedly higher IL 6 production compared with P4. High dimensional flow cytometry and unsupervised clustering converged on $CD14^+$ monocytes as the dominant IL 6 producing population, with drospirenone showing the strongest increase in both IL 6^+ cell frequency and per cell cytokine expression. Additional IL 6 producing subsets, including $CD123^+$ plasmacytoid and $CD123^-$ conventional dendritic cells, were also selectively activated by synthetic progestins. To determine whether these hormonal differences extend to adaptive immunity, naïve human $CD4^+$ T cells were differentiated under Th1 and Th2 polarizing conditions. Natural P4 suppressed Th1 differentiation and promoted Th2 lineage commitment, whereas synthetic progestins failed to reproduce these immunoregulatory effects and instead maintained or enhanced proinflammatory Th1 associated cytokine expression. Together, these findings identify $CD14^+$ monocytes as the principal cellular target of synthetic progestin induced IL 6 production and demonstrate that synthetic progestins disrupt P4 mediated regulation of naïve T cell differentiation. These data establish synthetic progestins as potent immune modulators that simultaneously dysregulate innate and adaptive immunity, providing a compelling mechanistic basis for the epidemiological association between oral contraceptive use and autoimmune disease susceptibility.

Tu113. Spatial Transcriptomic Profiling of Pancreatic Chemokine-immune Niches in Type 1 Diabetes

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Abstract Text: Type 1 diabetes (T1D) is characterized by immune-mediated destruction of pancreatic β cells, yet this process occurs in a spatially heterogeneous manner, with immune infiltration and β-cell loss varying across regions of the pancreas. Understanding how immune cell recruitment is organized within pancreatic tissue is therefore critical for defining the local microenvironments that support immune activation and persistence. Lymphotoxin signaling is well established in regulating chemokine programs that shape immune cell positioning and lymphoid tissue organization. Prior work from our group identified LTB (lymphotoxin-β), a central ligand in the lymphotoxin signaling pathway, as differentially expressed in pancreatic lymph nodes in T1D, suggesting dysregulation of lymphotoxin-associated pathways. However, whether these chemokine programs are spatially organized within pancreatic tissue, and how they contribute to localized immune recruitment in T1D, has not been defined. Given the role of lymphotoxin signaling in regulating chemokine expression, we sought to examine the spatial organization of chemokine programs within pancreatic tissue in T1D. To address this, we analyzed an integrated Xenium spatial transcriptomics dataset of formalin-fixed paraffin-embedded (FFPE) pancreatic tissue from 9 non-diabetic (ND), 4 autoantibody-positive (AAB^+), and 13 T1D deceased donors obtained through the Network for Pancreatic Organ Donors with Diabetes (nPOD). Samples were profiled using a ~5,000-gene panel, enabling broad interrogation of immune, stromal, and inflammatory pathways and facilitating spatial mapping of chemokine programs within the pancreatic microenvironment. Spatial mapping of gene expression revealed that chemokine

expression (e.g., CCL21, CXCL9, CXCL10, CXCL11) is spatially restricted to distinct regions and co-localizes with lymphatic endothelial cells and select immune cell subsets. In T1D, these chemokine signals exhibit altered spatial patterning, suggesting the presence of regionally defined immune niches that support localized immune cell recruitment within the pancreas. These findings underscore tissue heterogeneity within the pancreatic microenvironment and indicate that dysregulated chemokine programs shape immune niches of β-cell destruction in T1D.

Tu114. Dynamics of Autoreactome Remodeling Dictate the Durability of Response to Deep B-cell Depletion in Autoimmunity

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Abstract Text: Objective: Deep B-cell depletion via CAR T cell therapy and T cell engagers (TCEs) is emerging as a transformative treatment for autoimmune diseases, yet the durability of drug-free responses remains heterogeneous. We hypothesized that different B cell depletion strategies differentially affect the autoreactome, and that the extent of autoreactome remodeling might serve as a predictive biomarker for sustained immune reset and durability of response. Methods: Phage Immunoprecipitation Sequencing (PhIP-Seq; profiling ~20,000 human antigens) was performed on longitudinal sera from 27 patients with refractory autoimmune diseases treated with CD19 CAR-T (n=16; SLE=7, SSc=7, DM=2), low-dose blinatumomab (CD19 TCE; n=6; RA), or teclistamab (BCMA TCE; n=5; SSc, PSS, DM, RA), alongside 44 healthy controls. We quantified whole-autoreactome remodeling using Pearson correlation of fold-change profiles. Lower pre-to-post-treatment correlation indicates deeper disruption. Durability of response was stratified using Kaplan-Meier analysis. Results: Autoreactome profiles were highly individualized, with inter-individual Pearson r near zero among both patients and healthy controls (median r=0.01), confirming patient-specific autoantibody fingerprints. Post-treatment profiling revealed striking divergences in autoreactome remodeling. Teclistamab induced the most profound and immediate disruption (median pre-post r = 0.24). CD19 CAR-T drove a progressive, sustained remodeling deepening over time (median r=0.67 at 0–3 months, declining to r=0.55 at 3–6 months; p=0.009 vs baseline). Conversely, low-dose blinatumomab, leading to only transient B cell depletion, resulted in variable, less durable shifts (median r=0.67). Clinical disease trajectories mirrored autoreactome signatures: 14/16 CAR-T and 3/5 teclistamab patients achieved durable remission (mean follow-up 22 months), whereas all 6 RA patients treated with blinatumomab eventually developed flares (mean 5.4 months). Crucially, the magnitude of whole-autoreactome disruption was highly prognostic; among TCE-treated patients (n=11), achieving a remodeling threshold of r = 0.48 strongly predicted extended durability of response (log-rank p=0.012). Conclusions: PhIP-Seq unveils that patient-individual autoreactomes are differentially dismantled depending on the B-cell depletion approach. The profound restructuring achieved by teclistamab and CD19 CAR-T contrasts with the transient effects of low-dose blinatumomab, underscoring the effects of dosing and targeting plasma cells. Quantifying whole-autoreactome remodeling provides a novel, threshold-based biomarker predicting durability of response to deep B cell depletion, offering a critical tool for precision immunotherapy.

Tu115. Type 1 Diabetes Prevention Through Enhanced MHC–peptide Binding of an Insulin Epitope

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Abstract Text: Type 1 diabetes (T1D) is a chronic autoimmune disorder in which autoreactive T cells destroy pancreatic β cells. Both human studies and the NOD mouse model have identified multiple islet autoantigens, including insulin, IGRP, GAD, IA-2/ICA512, and hybrid peptides; however, distinguishing the primary initiating antigen from those arising via epitope spreading remains essential for designing early interventions. Multiple lines of evidence support insulin as a central initiating antigen: insulin autoantibodies arise early, particularly in young individuals, and correlate with T cell responses; insulin promoter VNTR polymorphisms reduce thymic insulin expression; and AIRE regulates thymic insulin presentation, with its deficiency permitting escape of high-affinity insulin-reactive T cells and increasing T1D susceptibility. The majority of pathogenic insulin-specific $CD4^+$ T cells in the NOD model are directed against the insulin B chain 9-23 epitope (InsB:9-23) presented by MHC class II allele I-A^g7 in a specific binding configuration termed register 3. In this arrangement, an arginine at position p9 interacts suboptimally with the MHC-binding pocket. Replacing this residue with glutamic acid improves peptide-MHC stability and enhances detection of InsB:9–23–specific $CD4^+$ T cells. These findings suggest that introducing this single amino acid substitution in the germline could enhance thymic presentation and promote central tolerance. CRISPR/Cas9-mediated genome editing was used to engineer the p9 position (R to E mutation) into one Ins2 allele while keeping Ins1 intact, generating heterozygous Ins2^R>E mice that show complete protection from both spontaneous and PD1-induced diabetes. This protection was mediated through increased thymic deletion of developing $CD4^+$T cells specific for InsB:9-23, consistent with enhanced presentation of this dominant epitope, and was disrupted in the setting of Aire deficiency, underscoring the requirement for Aire-dependent thymic expression. These results establish insulin as a primary driver of T1D initiation in the NOD model and demonstrate that enhancing thymic presentation at the level of a single peptide register is sufficient to reshape central tolerance and prevent disease. This work highlights targeted modification of self-epitopes as a promising antigen-specific strategy for disease prevention and provides a framework for next-generation tolerance-inducing therapies in T1D.

Tu116. Multimodal Analysis of S100A Proteins Suggests a Role in Immune-mediated Beta Cell Responses in Type 1 Diabetes

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Abstract Text: Objective: Type 1 Diabetes(T1D) is caused by autoimmune destruction of insulin-producing beta cells. We integrated *in vitro* and *in situ* tissue approaches to investigate the potential role of S100A1 proteins in T1D. Methods: Pancreatic tissue from a non-diabetic (ND) and three donors with T1D was stained with a high-plex16-marker panel acquired with Orion. Spatial image analysis with quantification of fluorescent signal and cells, throughout the entire tissue section and in regions of interest (ROIs) (islet, peri-islet, and exocrine) was performed with a semi-automated image analysis pipeline combining supervised and unsupervised machine learning based algorithms. Spatial transcriptomics in pancreas was investigated for 2T1D, 2ND, and 2 other diabetic cases. Additionally, we explored previously published pancreatic islet scRNAseq data from 27ND, 11AAB$^+$, 10T1D, and 17T2D. Isolated human islets and human islet organoids were cultured with proinflammatory cytokines (IFN-γ, TNF-α, IL-1β)$^{+/-}$S100A1$^{+/-}$HLA matched PBMCs. After culture with cytokines$^{+/-}$S100A1$^{+/-}$PBMCs, islet function was measured by glucose-stimulated insulin secretion (GSIS), and islets were subsequently stained with HLA-II, HLA-I,

Glucagon, TUNEL,CD45 and Insulin and acquired with the Stellaris5 confocal. Results: Pancreatic tissue data containing 1073 islets with more than 5 million cells from 4 nPOD donors was retrieved. Higher density of $S100^+$ cells was observed in T1D compared with ND in whole tissue and all ROIs by both supervised and unsupervised AI strategies. $S100^+$ cells created a cluster with $CD31^+$ cells, but not other immune cells were enriched in that cellular neighborhood. In the T1D patients, the percentage of $S100^+$ cells in the peri-islet regions correlated with a higher percentage of $insulin^+$ cells and a lower percentage of stressed HLA-II^+ beta cells in those islets. Transcriptomics results showed an increase in S100A10 and S100A1 in ductal cells in T1D tissue and in T1D activated stellate cells throughout scRNAseq when compared to ND. S100A1 didn't induce nor prevented from cytokine mediated hyperexpression of HLA-I, but we observed a modest reduction in islet immune infiltration and improvement in insulin secretion when S100A1 proteins were in co-culture with PBMCs. Conclusions: Our preliminary data suggests a protective role for S100 proteins in T1D. However, further investigations are needed to better understand the mechanisms and their specific role during immune mediated damage of beta cells.

Tu117. Microproteins as a Potential Source of Antigens in Autoimmunity

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Abstract Text: Objective: Microproteins are tiny proteins, typically smaller than 100 amino acids, often not recognized through conventional genome annotation and mislabeled as being non-functional. It was recently shown that peptide epitopes resulting from microproteins are presented on MHC complexes. However, the immune implications of microprotein expression are not well understood. We hypothesize that expression in the thymus presents a natural filter on microproteins, where those expressed in the thymus are tolerized and are, therefore, more likely to have broad tissue expression and function. On the other hand, microproteins not expressed in the thymus are likely to be restricted to immune-privileged tissues. Methods: To interrogate this in the context of Type 1 Diabetes(T1D), we first profiled the immunopeptidome and translatome of stem cell-derived pancreatic cells and created an epitope library containing ~58,000 epitopes. We utilize our lab's SABR antigen discovery platform to investigate whether islet-infiltrating T cell clones derived from T1D patients recognize micropeptide-derived epitopes. Results: Our initial screens identify candidate epitopes potentially recognized by islet-infiltrating T cells, which require further validation. Conclusion: Successfully identified peptides from the screen highlight the immunological relevance of microproteins. We will further compare how the thymic expression of micropeptides targeted by T cells correlates with their immunogenicity. Additionally, we plan to extend this research to other autoimmune conditions to explore the role of microproteins as sources of autoantigens.

Tu118. The Immunomodulatory Role of Bile Acids in Primary Sclerosing Cholangitis

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Abstract Text: Introduction: Primary sclerosing cholangitis (PSC) is a progressive liver disease characterized by chronic cholestasis, leading to inflammation and immune-mediated fibrosis of the intrahepatic and extrahepatic bile ducts. Patients with PSC often progress to cirrhosis and may ultimately require liver transplantation. Current management options are limited, and there are no FDA-approved therapies for PSC. Genome-wide association studies (GWAS) have identified strong associations between PSC and variants in human leukocyte antigen (HLA) genes, as well as other genes involved in the adaptive immune system, suggesting an autoimmune etiology. However, the exact pathophysiology of PSC remains unclear. One key area of interest is how prolonged exposure to high levels of bile acids during chronic cholestasis affects immune cell function, survival, and proliferation. Our project focused on taurine-conjugated bile acids, which are enriched in the bile acid pool of patients with PSC. Methods: $CD4^+$ and $CD8^+$ T cells isolated from wild-type mice were cultured in the presence of taurine-conjugated

bile acids (TCA and TCDCA). Live-cell imaging was used to assess viability and proliferation. Bulk RNA sequencing was performed to characterize transcriptional changes in bile acid–exposed T cells. Results: RNA sequencing revealed differential expression of several PSC susceptibility genes identified by GWAS, including Bcl2l11, Bach2, and Cd28, in bile acid–exposed T cells. Prior human studies have shown that risk alleles at these loci are associated with reduced gene expression in $CD4^+$ T cells and, in some cases, diminished protein function. Interestingly, bile acid exposure also downregulated the expression of these genes. These findings suggest that the cholestatic bile acid environment may impact pathways already vulnerable due to underlying genetic risk in PSC, highlighting a potential gene–environment interaction that warrants further study.

Tu119. Real-world Effectiveness and Immunophenotype-based Optimization of Belimumab Therapy in Active Lupus Nephritis: LOOPS Registry and FLOW Study

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Abstract Text: Objectives: Belimumab (BEL), a human monoclonal antibody targeting soluble B-cell-activating factor (BAFF), is effective against lupus nephritis (LN). This study assessed how BEL modifies the peripheral blood immunophenotypes and identified immunophenotypic markers predicting a favorable response to BEL in active LN. Methods: Patients with biopsy-proven ISN/RPS class III/IV LN treated with standard of care (SoC: glucocorticoids plus mycophenolate mofetil or cyclophosphamide) were analyzed. Those receiving BEL in addition to SoC (BEL^+SoC, n=40) were compared with SoC alone (n=35). Using flow cytometric analysis, we performed peripheral blood immunophenotyping to evaluate treatment effects and to identify predictors of complete renal response (CRR) at 52 weeks. Results: Baseline clinical characteristics and immunophenotypes were comparable between the groups. The BEL^+SoC group showed a higher CRR rate (SoC vs BEL^+SoC = 40% vs 68%, P=0.02) at 52 weeks. The BEL^+SoC group showed significantly greater reduction rates in $CD3^-CD19^+CD27^-IgD^+$ naïve B cells ($^+$63.1% vs −71.9%, P=0.01), $CD3^-CD19^+CD27^-IgD$- double-negative B cells ($^+$2.9% vs −44.6%, P< 0.01) and $CD3^-CD19^+CD20^-CD27^+CD38^+$ plasmablasts (-4.3% vs -93.2%, P=0.02) at 52 weeks. No baseline clinical factors were associated with CRR in either group. However, immunophenotyping revealed that increased pretreatment plasmablasts were associated with CRR in the BEL^+SoC group (P=0.04). Moreover, ROC analysis identified 2.2% of plasmablasts as the optimal cut-off predicting CRR, and patients with plasmablasts ≥2.2% achieved higher CRR rates within the BEL^+SoC group (< 2.2% vs ≥2.2% = 50% vs 93%, P=0.04). Conclusion: In induction therapy for active LN, BEL combination therapy was effective, supporting plasmablast-guided identification of patients achieving greater therapeutic benefit.

Tu120. Prediction for the Progression of Difficult-to-treat (D2T) Rheumatoid Arthritis by Machine Learning Scoring System, from the FIRST Registry

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Abstract Text: Objective: This study aimed to develop and validate a prediction model for future D2T RA progression and to support optimized use of b/tsDMARDs. Methods: This study used data from the FIRST registry (N=5,724), a multicenter cohort of RA patients initiating b/tsDMARDs. We included b/tsDMARD-naïve patients with inadequate csDMARD response, enrolled between 2013 and 2023. Patients were prospectively observed to determine whether they met D2T RA criteria. Predictive models for D2T RA progression were developed using machine learning from baseline characteristics. Furthermore, a simplified scoring system was created. Results: A

total of 1,221 patients were analyzed, of whom 193 (15.8%) progressed to D2T RA. These patients had higher tender joint counts, global assessment, patient's pain scale, HAQ, and CDAI, and more frequent coexisting lung diseases at 1st b/tsDMARDs initiation. Among the various machine learning models tested, Lasso logistic regression (AUC 0.71) identified pain scale, ESR, and coexisting lung disease as key predictors. The scoring system generated using these factors demonstrated comparable performance and allowed identification of patients at high-risk for D2T RA progression. Among these patients, those who received IL-6Ri as their first b/tsDMARD significantly reduced D2T RA progression (relative risk: 0.48 (0.25-0.91). Conversely, in the low-risk group, the first b/tsDMARDs class showed no significant impact on D2T RA progression. Conclusion: We developed and validated a scoring system predicting D2T RA progression and demonstrated the potential benefit of IL-6Ri as initial therapy in high-risk patients for D2T RA. This strategy may advance precision medicine in RA and help address unmet needs.

Tu121. Profiling of Autoreactive IGHV4-34^{+} B Cells in Systemic Lupus Erythematosus Through Single-cell B Cell Receptor Analysis

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Abstract Text: Systemic lupus erythematosus (SLE) is characterized by the production of autoantibodies. The diversity of B cell receptors (BCRs) is generated by V(D)J recombination and class switching. However, the mechanisms underlying the production of autoantibodies are not fully elucidated. We aimed to uncover the process, especially focusing on IGHV4-34, known as one of the autoreactive BCRs. Peripheral blood mononuclear cells from 81 SLE patients and 24 health controls (HCs) were subjected to scRNA-seq, CITE-seq, and BCR-seq. For more detailed BCR analysis, CD19^{+} B cells from 15 SLE patients and 2 HCs were analyzed as well. Xenium-based spatial transcriptomics analyses were performed with renal tissues from 3 SLE patients. The proportion of atypical B cells (ABCs) and plasmablasts was significantly increased in SLE ($p< 0.05$ for both). Cell-cell communication analysis demonstrated that BAFF (B-cell Activating Factor) signal from dendritic cells and monocytes was upregulated in SLE ($p< 0.05$), affecting memory B cells and plasmablasts. The usage of IGHV4-34 in ABCs was higher than that in unswitched memory B cells and switched memory B cells ($p< 0.05$ for both), suggesting that ABCs represent a potential source of IGHV4-34^{+} plasmablasts. The proportion of IgG^{+}IGHV4-34^{+} ABCs positively correlated with SLEDAI-2K ($p< 0.05$). In renal tissues of lupus nephritis, IgG1^{+}IGHV4-34^{+} plasma cells infiltrated into perivascular and periglomerular regions. In summary, multimodal analyses revealed the dynamics of IGHV4-34^{+} B cells in SLE. These findings suggest the pivotal role of IGHV4-34^{+} B cells in the pathogenesis of SLE and highlight them as a potential biomarker and therapeutic target.

Tu122. UNIChro-seq Enables Efficient Functional Screening of Rheumatoid Arthritis Risk Alleles

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Abstract Text: Background: Genome-wide association studies (GWAS) have identified hundreds of genetic variants associated with rheumatoid arthritis (RA), the majority of which reside in non-coding regions and are presumed to act through gene regulatory mechanisms. However, conventional quantitative trait locus (QTL) analyses often fail to detect functional effects for many RA risk variants due to limited statistical power, reliance on naturally occurring alleles, and an inability to capture context-dependent regulatory dynamics. These limitations obscure the causal interpretation of disease-associated variants and hinder mechanistic insights into RA pathogenesis. Methods: To overcome these challenges, we developed UNIChro-seq, a high-sensitivity chromatin accessibility profiling platform

based on a modified ATAC-seq framework. UNIChro-seq selectively enriches specific chromatin-accessible regions, quantifies allelic imbalance using unique molecular identifiers (UMIs), and enables highly multiplexed pooled library preparation. Applying this approach to heterozygous loci and CRISPR-edited alleles in human primary cells, we assessed context-dependent effects of RA variants on chromatin accessibility across diverse stimulation conditions. Results: Primary $CD4^+$ T cells from five healthy donors were stimulated over a 72-hour time course and sampled at 12 distinct time points. Among 20 fine-mapped RA risk variants tested, nine exhibited significant caQTL effects, including two variants with stimulation time–dependent dynamics that were undetectable by conventional approaches. Notably, an intronic variant within the LEF1 locus (rs58107865), fine-mapped with the highest posterior probability, displayed subset-specific caQTL effects. The RA risk allele reduced chromatin accessibility at baseline, with attenuation upon stimulation, and this directionality was validated using genome-edited $CD4^+$ T cells. Consistent with these findings, the rs58107865 risk allele correlated with reduced LEF1 expression in naïve $CD4^+$ T cells. Furthermore, single-cell multiomics of LEF1 knockdown T cells revealed that reduced LEF1 expression was associated with an increased abundance of activated naïve $CD4^+$ T cells, suggesting altered activation thresholds as a potential disease-relevant mechanism. Conclusions: UNIChro-seq enables sensitive detection of caQTL effects from a limited number of donors. By bridging the gap between GWAS signals and functional regulatory mechanisms, this approach provides a scalable framework for elucidating the genetic and epigenetic architecture underlying RA risk and other complex immune-mediated diseases.

Tu123. Increased Uric Acid Degradation Attenuates Psoriasiform Dermatitis by Suppressing the Mitochondrial $Ca2^+$–mtROS–CCL20–IL-17A Axis

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Abstract Text: [Background] Psoriasis is driven by the IL-23/IL-17 axis, and psoriatic epidermis exhibits accelerated turnover with increased purine degradation and elevated uric acid (UA) production. Although UA is recognized as a pro-inflammatory DAMP, whether UA and its metabolic pathway affect psoriatic skin inflammation remains unclear. This study investigated the effects of increased UA degradation on psoriasiform dermatitis. [Methods] We used imiquimod (IMQ)-induced psoriasiform dermatitis in wild-type (WT) mice and uricase-overexpressing transgenic mice (UOXTg), a model of increased UA degradation and reduced tissue UA levels. Disease severity, cytokine expression, and IL-17A–producing T Cell infiltration were evaluated. Primary mouse keratinocytes were stimulated with UA and IMQ to assess CCL20 induction and mitochondrial responses. [Results] UA levels in WT lesional skin increased markedly after IMQ application, whereas this rise was suppressed in UOXTg mice. UOXTg mice displayed reduced PASI scores, decreased epidermal thickness, and lower IL-17A and CCL20 expression in skin lesions. Because CCL20 recruits $CCR6^+$IL-17A–producing γδT cells, we assessed T Cell infiltration and found reduced IL-17A–producing γδT cells in UOXTg lesions, with no differences in lymphoid organs. In keratinocytes, UA enhanced IMQ-induced CCL20 expression and induced mitochondrial $Ca2^+$ overload and mitochondrial reactive oxygen species (mtROS). Inhibition of mtROS suppressed UA-mediated CCL20 upregulation. *In vivo*, UOXTg skin showed reduced mitochondrial oxidative stress, with decreased mtDNA damage and lower 8-OHdG levels compared with WT. [Conclusion] Increased UA degradation attenuates psoriasiform dermatitis by limiting mtROS-dependent CCL20 induction and IL-17A–producing γδT cell recruitment. UA handling and mitochondrial oxidative stress represent potential therapeutic targets in psoriasis.

Tu124. Integrating Transcriptomic and Clinical Data to Predict Disease Progression in Sjögren's Syndrome

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Abstract Text: Sjögren's syndrome (SS), the second most common autoimmune rheumatic disease, is characterized by lymphocytic infiltration of exocrine glands causing oral and ocular dryness. SS treatment predominantly remains palliative. The multicentre, randomized, double-blind, placebo-controlled Trial of Anti–B cell Therapy in Patients with Primary SS (TRACTISS, 65360827) evaluated whether two courses of rituximab improved symptoms in 133 patients over two years (Bowman et al., 2017). From the TRACTISS cohort, 52 patients were simultaneously enrolled in a T cell transcriptomics sub-study, with samples collected at baseline, weeks 16 and 48. These samples were used to identify transcriptional signatures associated with the composite of relevant endpoints for SS (CRESS) assessed at weeks 24 and 48. We generated a low dimensional representation of these transcriptomes at the three timepoints using multi-omics factorial analysis (MOFA). MOFA is a computational method for discovering the principal sources of variation (both biological and technical) in multi-omics data sets in an unsupervised manner (Argelaguet et al., 2018). This MOFA model explained 60% of the variation in the dataset and identified 15 latent factors (LF), each mathematically defined as a linear combination of the input genes. One LF, LF10, was associated with CRESS assessed at week 24. Gene set enrichment and variation analysis (GSEA, GSVA) revealed that a T cell exhaustion signature was enriched on LF10. To identify the origin of this T cell exhaustion signature we performed an additional GSVA on transcriptomic data from sorted CD4 and CD8 T cells collected at baseline. Only CD4 T cell exhaustion predicted CRESS assessed at weeks 24 and 48. Specifically, patients with higher expression of genes associated with CD4 T cell exhaustion at baseline were less likely to show a clinical response. Our findings hence provide insights into biomarkers and molecular mechanisms underlying SS progression.

Tu125. Age-related Hormonal Signaling Defines a Pathogenic Monocyte Subset in Giant Cell Arteritis

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Abstract Text: Giant cell arteritis (GCA) is an inflammatory vasculitis affecting large and medium-sized vessels with an exclusive onset in individuals over 50 years of age. To date, disease-specific diagnostic biomarkers are lacking. The inflammatory response is acute in nature and largely driven by the innate immune system. Histopathological analyses reveal extensive infiltration of affected arteries by mononuclear phagocytes, including monocytes and macrophages, underscoring a central role of innate immune mechanisms in GCA pathogenesis. However, whether distinct pathogenic innate immune cell subsets contribute to disease initiation remains unclear. Here, we performed single-cell transcriptomic profiling of circulating monocytes from peripheral blood of patients with active, untreated GCA and age-matched healthy controls. This approach identified a distinct monocyte population within the classical monocyte compartment that was exclusively present at the time of initial diagnosis and absent in healthy individuals. Notably, this disease-associated subset was no longer detectable three months after initiation of therapy, suggesting involvement in early disease stages. Transcriptomic characterization revealed enrichment of sex hormone–responsive gene expression programs, with a pronounced activation of estrogen-associated signaling pathways. Given that systemic estrogen levels undergo substantial alterations around the age of 50, these findings suggest a potential link between age-related hormonal changes and the emergence of this pathogenic monocyte population. Collectively, our data identify a previously unrecognized, hormone-responsive monocyte subset with potential relevance for early disease mechanisms, biomarker development, and therapeutic targeting in GCA.

Tu126. Selective, Covalent, Oral IRF5 Inhibitors Pharmacologically Modulate Immune Responses in Rodents and in Human Peripheral Blood Mononuclear Cells from Patients with Systemic Lupus Erythematosus

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Abstract Text: Interferon regulatory factor 5 (IRF5) is a genetically and mechanistically validated driver of autoimmunity in diseases with unmet medical needs, making it an attractive target for pharmacological intervention. Objectives: The research aimed to discover small molecule inhibitors of IRF5 and characterize the biological and pharmacological activity of initial hits and optimized molecules. Methods: IRF5 was screened in OmniDEL - a proprietary DNA-encoded library platform. Compounds were characterized in human THP-1 monocytes, in human primary peripheral blood mononuclear cells (PBMCs), and in mouse models of acute inflammation. Activation with the TLR7/8 agonist, R848, led to nuclear translocation of IRF5 resulting in secretion of type I interferons and pro-inflammatory cytokines. Results: Selective covalent small molecule inhibitors of IRF5 were identified by OmniDEL. Subsequent molecules were optimized for increased cell permeability, potency, and metabolic stability. Compounds inhibited nuclear translocation of IRF5 as well as downstream production of type I interferons and pro-inflammatory cytokines in PBMCs from healthy donors. A similar effect was seen in PBMCs from systemic lupus erythematosus (SLE) patients, with and without TLR7/8 agonism. *In vivo*, the compounds attenuated TLR7/8-induced interferon and cytokine responses in mice after oral dosing. Conclusions: This work presents discovery of covalent small molecule IRF5 inhibitors that are pharmacologically active *in vitro* and *in vivo*. The molecules modulated type I interferon and cytokine secretion in PBMCs from healthy donors as well as from patients with SLE. These findings provide initial validation that oral small molecule inhibitors of IRF5 hold promise in autoimmune and other serious inflammatory diseases.

Tu127. Defining Rheumatoid Arthritis Endotypes and Treatment Response Profiles Through Serum Proteomics and Longitudinal Disease Trajectories

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Abstract Text: Despite advances in therapeutics, treatment failure rates in rheumatoid arthritis (RA) remain high. Due to pronounced disease heterogeneity, patients with similar clinical manifestations can exhibit widely varying treatment responses and prognoses. Molecular disease endotyping has emerged as a promising strategy to dissect disease heterogeneity and enable precision medicine. In this study, we leveraged serum proteomics from 219 RA patients with early or established disease to define molecular endotypes and evaluated their associations with clinical features and response to conventional synthetic and biological disease-modifying antirheumatic drugs (DMARDs). We performed unsupervised clustering on the baseline proteome data and identified six endotypes with distinct molecular characteristics. These endotypes exhibited a continuum of inflammatory activity, with early disease endotypes showing minimal inflammation compared to those associated with established disease. Treatment response analysis revealed endotypes enriched for responders or nonresponders to DMARDs overall, as well as to specific therapies. Further characterization of their clinical and synovial tissue phenotypes revealed complex clinical and molecular interactions. While baseline clinical disease activity showed minimal differentiation across endotypes, we observed a consistent stratification among endotypes defined by longitudinal disease trajectories and molecular profile. Gene set signature analysis of synovial tissue samples further suggested shift in macrophages and T Cells enrichment across endotypes, highlighting opportunities to further investigate the relationship between synovial

tissue pathotypes vs. systemically defined endotypes. We also evaluated the impact of treatment on endotypes by building a machine learning (ML) model that predicts endotypes from post-treatment samples. This model utilized a concise feature set and nonetheless demonstrated strong predictive performance. Comparing pre- and post-treatment endotypes within the same patient revealed varying levels of shifts, indicating complex dynamics in disease states pertaining to treatment response and progression. Together, these findings highlight the potential of proteomics-based molecular endotyping to identify responder-enriched patient subpopulations and provide a ML model for future research on targeted proteomics for endotyping. Furthermore, this work offers insights into biomarker profiles and clinical characteristics associated with treatment response, supporting the development of precision medicine strategies in RA.

Tu128. Insulin-reactive B Cells Are Found in Early-stage Type 1 Diabetes Individuals Without Requirement for Insulin Autoantibody Seropositivity or Somatic Hypermutation

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Abstract Text: The presence of two or more islet autoantibodies classifies early-stage type 1 diabetes (T1D), but islet autoantibodies, including insulin autoantibodies, can be transient. To adequately survey ongoing B cell insulin autoimmunity as a potential T1D biomarker, we used single-cell multi-omics and hybridoma technology to isolate anti-insulin B cell receptors (BCRs) from early stage T1D individuals. We identified n= 46 ELISA-validated anti-insulin BCRs from the peripheral blood of n = 19 TrialNet Pathway to Prevention participants who were positive for ≥ 2 islet autoantibodies, with either normal (Stage 1 T1D) or impaired (Stage 2 T1D) oral glucose tolerance. Of the individuals who harbored anti-insulin B cells, 32% screened negative for insulin autoantibody at the time of blood draw. For the anti-insulin BCRs in which B cell subset identity was known, 7/17 were isolated from atypical memory B cells. Anti-insulin BCRs expressed diverse variable heavy chain (VH) gene segments, with the autoreactive-prone VH4-34 gene expressed most prevalently (15% of BCRs). Additionally, 74% of anti-insulin BCRs were IgG, most of which expressed IgG3. Anti-insulin BCR somatic hypermutation ranged; 52% of anti-insulin BCRs exhibited no VH gene amino acid substitutions, whereas 17% contained >10 amino acid substitutions. Germline reversion of many mutated anti-insulin BCRs did not abolish insulin binding. These findings show circulating anti-insulin B cells can persist in the absence of insulin autoantibodies and suggest B cells expressing specific variable genes hold increased potential to bind insulin. This provides a foundation for future B cell biomarker development to evaluate T1D disease progression and response to immunotherapy.

Tu129. Profiling Autoantibodies in Thyroid Eye Disease and Graves' Disease Using a Proteome-wide Screening Approach

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Abstract Text: Thyroid Eye Disease (TED) is an autoimmune condition characterized by orbital inflammation, fibroblast activation, and tissue remodeling, most commonly arising in the context of Graves' disease (GD). Although thyroid-stimulating hormone receptor (TSHR) autoantibodies define GD, they do not fully explain the immunological mechanisms that lead to orbital involvement. Previous studies have identified autoantibodies against a focused set of orbital antigens, notably calsequestrin and collagen XIII, highlighting the presence of diverse autoreactivities in TED and raising important questions about the pathogenic significance of surface versus intracellular antigen targets, as well as whether such autoantibodies reflect causal pathways, disease amplification, or secondary responses to tissue injury. Motivated by these observations, we hypothesized that TED may involve a substantially broader autoantibody repertoire than previously characterized, and that an unbiased, high-content screen could

uncover novel reactivities with relevance to disease biology. To test this, we applied a HuProt microarray platform (>21,000 human proteins) to profile serum autoantibodies from over 100 TED patients, spanning both acute and chronic disease presentations. A smaller cohort of individuals with GD was analyzed in parallel, together with healthy controls, to provide exploratory comparative context. Proteome-wide profiling revealed distinct autoantibody signatures that differentiated TED samples from controls and further highlighted heterogeneity within the TED cohort. These findings extend prior candidate-based and immunoproteomic studies by delivering the first comprehensive, unbiased survey of autoantibody repertoires in TED. The resulting antigen candidates may inform mechanistic hypotheses, support future risk-stratification efforts, or guide diagnostic development. Interpretation of these data incorporates the known strengths and limitations of large-scale protein microarrays and the ongoing debate regarding the mechanistic relevance of intracellular versus surface-exposed targets. Disclosures: All authors are employees and stockholders of Amgen Inc.

Tu130. CXCL12hi Sublining SF Involved in Treatment Resistance for Cytokine-blocking Agents via Senescent-like Property

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Abstract Text: Rheumatoid arthritis (RA) patients show heterogeneous responses to disease-modifying anti-rheumatic drugs (DMARDs), with approximately 40% exhibiting resistance to biologic or targeted synthetic DMARDs. This study aims to elucidate one of the mechanisms contributing to treatment resistance in Japanese RA patients. Synovial samples were collected from 54 RA patients, who fulfilled ACR/EULAR 2010 rheumatoid arthritis classification criteria. We analyzed synovial tissues by flow cytometry (FCM) and single cell RNA-sequencing (scRNA-seq), and performed integrated analysis with clinical information and spatial transcriptomics and proteomics profiling. By hierarchical clustering of cell types identified by FCM and scRNA-seq, we identified a synovial cell module composed of CXCL12hi gene X^+ sublining synovial fibroblasts (SF) and plasmablasts that was associated with treatment resistance to cytokine-blocking agents. Spatial multi-omics analysis revealed close proximity and communication between CXCL12hi gene X^+ sublining SF and plasmablasts via the CXCL12–CXCR4 axis. CXCL12hi gene X^+ sublining SF expressed CXCL12 the highest among all SF clusters, and CXCL12 protein distribution was significantly correlated with plasmablast's density. Furthermore, *in vitro* knock-down experiments demonstrated that gene X is essential for the expression of senescent cancer associated fibroblast (CAF) -related genes including CXCL12 in RA-SF. Senolytic drug showed an additive treatment effect to Etanercept on collagen antibody–induced arthritis. These findings suggested that CXCL12hi gene X^+ sublining SF exhibits senescence-like features and promotes plasmablast persistence leading to treatment resistance to cytokine-blocking agents. Synovial multi-omics analyses indicated that senescent fibroblast may explain one of the pathologies associated with treatment-resistant synovitis.

Tu131. ANB033, a Novel CD122 Antagonist in Development for the Treatment of Inflammatory and Autoimmune Diseases, Inhibited IL-15 and IL-2 Signaling in $CD8^+$ and $CD4^+$ T Cells *in vitro* and in a Murine Model of GvHD

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Abstract Text: CD122 is the beta subunit of the receptors for both IL-15 and IL-2. It regulates cytotoxic $CD8^+$ T, memory $CD4^+$ T, and natural killer (NK) cell survival, proliferation, and differentiation. In inflammatory or autoimmune diseases, inflammation driven by IL-15 and IL-2 overexpression can lead to excessive CD122 signaling and the persistence of pathogenic $CD8^+$ T, $CD4^+$ T, and NK cells; therefore, CD122 represents a promising therapeutic target. ANB033 is a novel CD122 antagonist monoclonal antibody engineered to bind to an optimal epitope on CD122 with high affinity, resulting in potent inhibition of IL-15 and IL-2 signaling. *In vitro*, ANB033 inhibited IL-15- and IL-2-mediated STAT5 signaling in cytotoxic $CD8^+$ T cells, memory $CD4^+$ T cells, and NK cells, leading to downstream inhibition of proliferation and inflammatory cytokine production. ANB033 was evaluated in a xenogeneic graft-versus-host disease (X-GvHD) model, which reflects pathogenic mechanisms driven by activated $CD8^+$ and $CD4^+$ T cells. Mice treated with ANB033 demonstrated potent reduction of human $CD8^+$ and $CD4^+$ T cell numbers, reduced body weight loss, and a greater survival benefit compared to isotype control or belatacept (CTLA-4 Ig). Additionally, mice treated with ANB033 exhibited prolonged survival beyond the treatment period. ANB033 significantly decreased GvHD-related pathology in the murine model and may provide therapeutic value in T cell mediated inflammatory and autoimmune diseases where IL-15 and IL-2 are pathogenic drivers of disease. These data support the rationale for evaluating ANB033 in an ongoing Phase 1b study in celiac disease.

Tu132. Using Pathogenic CD8 T Cells to Identify HLA-$B27^+$ Axial Spondyloarthritis and Acute Anterior Uveitis

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Abstract Text: Axial spondyloarthritis (axSpA) and acute anterior uveitis (AAU) are chronic inflammatory diseases strongly associated with HLA-B27 allele. However, a lack of specific diagnostic biomarkers often leads to delayed diagnosis and poor outcomes. Although HLA-B27 is central to pathogenesis by presenting arthritogenic peptides to CD8 T cells, its low positive predictive value limits its utility as a standalone diagnostic tool. While pathogenic CD8 T cells have been identified within the inflamed tissues of patients, their presence and enrichment in the peripheral circulation is unknown. We hypothesized that cross-reactive, pathogenic CD8 T cells are enriched in the peripheral blood of patients and represent a potential diagnostic tool. PBMCs from HLA-$B27^+$ adults with axSpA/AAU (n=33) and healthy controls (n=45) were analyzed using dual tetramer-based flow cytometry. To identify the cross-reactive, pathogenic T cells, we utilized a synthetic peptide pool with high affinity for disease-associated TCRs and YeiH, a Gram-negative bacterial protein. Pathogenic cells were defined by dual binding to both synthetic peptide and YeiH tetramers; non-pathogenic cells bound YeiH only. An AAU-derived TCR and influenza-specific TCR served as validation controls. Patients demonstrated significantly higher frequencies of pathogenic CD8 T cells than controls (median 0.0100% vs 0.0016%, $p < 0.0001$). ROC analysis demonstrated strong diagnostic potential (AUC 0.87, 95% CI 0.79-0.95). Conversely, non-pathogenic YeiH-specific cell frequencies did not differ between two groups, indicating the diagnostic signal is specific to the cross-reactive TCR repertoire rather than general microbial exposure. Pathogenic, cross-reactive CD8 T cells are significantly enriched in the peripheral blood of HLA-$B27^+$ axSpA/AAU patients, supporting their role in disease pathogenesis and linking molecular mimicry to T cell-mediated inflammation. Future studies will explore their functional properties and relevance in other HLA-$B27^+$ disease cohorts, which will lay the groundwork for a highly specific and reliable clinical diagnostic assay.

Tu133. Type I Interferons Are Required for Murine Roseolovirus-induced Autoimmune Gastritis

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Abstract Text: Human Roseoloviruses (HHV) have been associated with several autoimmune diseases notably encephalitis, multiple sclerosis (MS), connective tissue diseases and drug reaction with eosinophilia and systemic symptoms (DRESS). Due to high species-specific tropism, human herpesviruses are difficult to study in a controlled environment. Recently however, the murine roseolovirus (MRV) was sequenced and found to be a close homolog of HHV-6 and 7. MRV is a thymotropic virus that causes transient, severe thymic atrophy reducing mainly $CD4^+$ single positive (SP) thymocytes as well as double positive (DP) thymocytes. Our lab has recently established a unique model to study the interaction between HHVs and autoimmune disease wherein we show that neonatal infection with MRV causes autoimmune gastritis (AIG) in adulthood. We showed that $CD4^+$ T Cells are both required and sufficient for AIG development in our model. We also showed significant reduction of AIRE transcripts in medullary thymic epithelial cells (mTECs) of infected mice, and development of autoantibodies to several tissue antigens targeting almost all organ systems. Single cell RNA sequencing data showed that $CD4^+$ SPs, DPs and double negative (DN) thymocytes as well as mTECs can support productive infection of MRV. Interestingly, type I IFN signaling is disrupted in all infected cells. The goal of this study is to understand the mechanism of auto-immune gastritis development in the setting of neonatal MRV infection. Herein, we show that IFN signaling is involved in controlling MRV infection. We also show that MRV-induced type I IFN signaling is required for AIG development in infected mice. In fact, our data demonstrates that sterile type I IFN induction or infection by MCMV, another thymotropic virus causes thymic atrophy but not AIG, suggesting an MRV-specific mechanism of immune dysregulation. Using a unique animal model of virus-induced autoimmunity, our study sheds light on a novel type I IFN-dependent axis by which tolerance and thymopoiesis are disrupted neonatally.

Th164. Quantification of Sacroiliac Joint Inflammation by Total Body 18F-FDG PET Imaging: A Paradigm Change for Quantitative Assessment of axSpA

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Abstract Text: Although different scales exist to quantify sacroiliitis/BME by MRI; these scales are semi objective, and imaging must be done with similar scanners which limit their use in clinical studies. Total-Body (TB)-PET/CT is a novel scanner which captures simultaneously head-to-toe tracer distribution across all the body tissues/organs in a single imaging position. The objective of this study was to apply high-sensitivity TB-PET/CT imaging with 18F-FDG to quantify the degree of inflammation in SI joints in patients with Axial Psoriatic Arthritis (AxPsA). Methods-Using propensity score matching for sex, age, and BMI, 16 participants with an established diagnosis of psoriatic arthritis (PsA) and clinical history of inflammatory back pain (IBP) (PsA^+IBP) were compared with 12 participants with non-inflammatory [osteoarthritis] low-back pain, under the age of 40 years (to minimize age-related sacroiliac joint [SIJ] changes). All subjects underwent a single-timepoint 18F-FDG TB-PET/CT using 1/5th the standard radioisotope dose (78.2±4.9 MBq)(Abdelhafez Y,et al.J Nucl Med. 2022(10):1579-1585). Degree of inflammation was quantified by using maximum standardized 18F-FDG uptake value ratio corrected by the background level (rSUVmax). Results-TB-PET/CT imaging allowed to quantify the total spinal inflammatory load in each subject both in controls and in patients with PsA^+BP. rSUVmax were significantly higher in PsA^+IBP (n=16) compared to controls (n=12) ($p<.001$) . Axial involvement: The majority of PsA^+IBP participants (75%) demonstrated PET-evidence of spinal enthesitis including. Sacroiliitis-5/16(31%) patients with PsA^+IBP had concurrent active sacroiliitis evidenced qualitatively by higher 18F-FDG accumulation (2 unilateral and 3 bilateral with asymmetric uptake). In 5 patients, 10 SI joints were quantified for degree of inflammation. Quantitatively, the summed rSUVmax for the SI joints was 3.5 ± compared to .8± in the control group osteoarthritis patients ($p<.001$) Conclusion-TB-PET/CT imaging will be a paradigm change for quantitative assessment of axSpA and identifying non-radiographic axial SpA. Quantification of the total inflammatory burden (rSUVmax) of the SI joints demonstrating the ability of TB-PET/CT to quantify the

inflammatory load thus to have a numerical measure of the disease activity. This could be a unique tool (i) for quantifying the degree of disease severity (ii) for drug development; to determine the degree of inflammation before treatment and after treatment.

Tu135. Intranodal Administration of Vitamin D3-tolerogenic Dendritic Cells in Patients with Multiple Sclerosis: Results from a Phase I Dose-escalation Clinical Trial

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[1]FUNDACIÓ INSTITUT D'INVESTIGACIÓ EN CIÈNCIES DE LA SALUT GERMANS TRIAS I PUJOL (IGTP) - Dept of cell biology, Physiology, Immunology, UAB - FCE UAB- Barcelona, Siena, Toscana, Italy, [2]Hospital Germans Trias i Pujol, [3]University of Antwerp

Abstract Text: Background: Multiple sclerosis (MS) is a chronic autoimmune disease driven by aberrant T Cell responses against myelin antigens. Current immunomodulatory treatments provide non-specific immunosuppression with significant side effects. Tolerogenic dendritic cells (tolDC) represent promising antigen-specific immunotherapy aimed at restoring immune tolerance. We conducted a phase I, open-label, dose-escalation clinical trial (TOLERVIT-MS; EudraCT 2015-003541-26, NCT02903537) to evaluate the safety, tolerability, and preliminary efficacy of autologous vitamin D3-conditioned tolDC (VitD3-tolDC) loaded with myelin peptides, administered intranodally to patients with active relapsing-remitting MS. Methods: Eleven patients were enrolled across three dose cohorts (5, 10, and 15×10^6 cells), plus an additional cohort receiving 15×10^6 cells combined with immunomodulatory treatment. Patients received six intranodal injections, (four separated by 2 weeks and two by four weeks) and underwent clinical (EDSS, relapse rate), radiological (MRI with gadolinium-enhanced lesions), and immunological monitoring. Peripheral blood mononuclear cells were analyzed by flow cytometry for T Cell subpopulations and regulatory T cells (Tregs). Serum cytokine profiling was performed using Olink proteomics, and single-cell RNA sequencing coupled with T Cell receptor sequencing (scRNA-seq + TCR-seq) by XXX Results: VitD3-tolDC therapy was feasible, well-tolerated, with no serious adverse events or therapy-related reactions. The highest-dose cohort showed a trend toward reduced radiological activity, including decreased gadolinium-enhancing lesions. Immunological analyses revealed a trend toward increased Th2 responses and decreased pro-inflammatory Th17 subpopulations. Notably, flow cytometry demonstrated a statistically significant increase in $CD25^+CD127^-$ Tregs. Olink analysis identified changes in circulating cytokines. Currently scRNA-seq data is under analysis to elucidate treatment-induced transcriptional and clonal T Cell dynamics. Conclusions: Intranodal VitD3-tolDC immunotherapy is safe and shows promising immunomodulatory effects in MS patients, with significant Treg expansion. These findings support progression to a phase II efficacy trial, currently approved by regulatory agencies in Spain and Belgium.

Tu136. Autoreactivity to Secreted Proteins in Type 1 Diabetes

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Abstract Text: Type 1 diabetes (T1D) is an autoimmune disease that is characterized by immune-mediated destruction of insulin-producing beta cells. Despite decades of evidence that viral infections are linked to increased risk of T1D, the immune factors that may predispose some individuals to autoimmunity following infection are not fully explored. These factors may include autoantibodies that influence immune activity or regulation. To investigate this, we designed a custom yeast surface display library containing 1,517 proteins, focused on human secreted and/or extracellular factors. This library was used to investigate autoimmune repertoires from both T1D and healthy individuals. Analysis of a pilot cohort (n=238 cases, 84 controls) surfaced numerous autoreactivities present in cases but not controls, including proteins related to control of viral infections. Experiments to orthogonally validate these

findings are underway, in addition to expanding the cohort size. These findings will establish a foundation of T1D-related autoreactivity to secreted proteins relative to healthy individuals, and specific autoreactivities may suggest mechanistic linkages underlying infectious triggers of T1D.

W102. Loss of Tolerance to Perilipin-1 in a Mouse Model of Adipose Autoimmunity

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Abstract Text: Acquired generalized lipodystrophy (AGL) is characterized by progressive loss of adipose tissue and severe metabolic dysfunction, with clinical and histologic features suggestive of immune-mediated pathology. We recently identified autoantibodies against the lipid droplet protein perilipin-1 (Plin1) in patients with AGL, as well as in humans and mice deficient in AIRE, a key regulator of central tolerance. Aire-deficient mice develop marked mononuclear infiltration of white adipose tissue (WAT), resembling the panniculitis observed in AGL. We hypothesized that impaired central tolerance in the absence of AIRE permits the emergence of pathogenic T cell responses targeting adipose tissue. Using adoptive transfer and genetic approaches, we demonstrate that adipose inflammation in Aire-deficient mice is T cell–dependent. To define disease-associated T cell populations, we performed paired single-cell RNA and TCR sequencing of WAT-resident T cells. Aire deficiency was associated with an increased frequency of effector $CD4^+$ T cells, including a Th1-like population enriched for expanded clonotypes with shared CDR3α and CDR3β motifs, consistent with antigen-driven selection. Functional analysis of representative TCRs using a Plin1-spanning peptide library revealed specificity for multiple Plin1 epitopes. Epitope-specific tetramers confirmed the presence of activated Plin1-reactive $CD4^+$ T cells in WAT and draining lymph nodes of Aire-deficient mice. Notably, TCRs reconstructed from adipose-resident Tregs in wild-type mice recognized distinct Plin1 epitopes, suggesting that tolerance to Plin1 is a key component of adipose immune homeostasis. Together, these findings establish Aire-deficient mice as a model of adipose-directed autoimmunity and support a mechanism in which defective central tolerance to Plin1 enables the escape and activation of autoreactive T cells that drive adipose inflammation

W103. A Surviving Beta Cell Subpopulation Enriched in Patients with Type 1 Diabetes

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Abstract Text: Type 1 diabetes (T1D) is characterized by the autoimmune destruction of pancreatic beta cells. While most beta cells are lost, a subset of beta cells persists years and even decades after disease onset. Studying these cells is challenging as pancreatic biopsies are not performed on living donors, and thus how these particular cells escape immune killing remains poorly understood. Here, we applied a gene regulatory network module-based clustering approach on public islet scRNAseq data from 55 cadaveric donors: 12 with T1D, 11 at elevated risk for T1D due to autoantibodies (AAb^+), and 32 with no diabetes (ND). This approach identified a novel beta cell population enriched in donors with T1D (median frequency = 0.153 of all beta cells) compared to ND donors (median frequency = 0.037, FDR = 0.002 vs T1D) and AAb^+ donors (median frequency = 0.027, FDR = 0.002 vs T1D). These cells are defined by increased activity of transcription factors with well-characterized roles in beta cell survival, most notably IRF1, as well as BCL6, ETV6, CEBPD, ETS2, and JUNB. We found increased expression of immunomodulatory genes (e.g. SOCS1/3, HLA-E), stress response genes, and a subset of interferon-stimulated genes in this cluster. Several enriched genes have known variants associated with T1D (e.g. IRF1, SOCS1, IKZF4). We also observed evidence of dedifferentiation in these cells, with decreased expression of beta cell lineage-defining factors (MAFA, MAFB), autoantigens (PTRPN, IAPP, SLC30A8, G6PC2), and secretory genes. We confirm these features using independent public data from primary human beta cells stimulated with inflammatory cytokines *in vitro*. We also identify a similar population of alpha cells *in vivo*, supporting cytokine sensing as the driver of this

phenotype. Overall, we propose that this beta cell subset represents a resilient cell phenotype, and that promoting this naturally occurring program in beta cells may have important positive implications for T1D prevention and reversal.

W104. Lesional and Perilesional Tissue Comparisons Across Immune-mediated Inflammatory Diseases Reveal Disease-specific Immunoregulatory Mechanisms Mediated by Peripheral Helper and Regulatory T Cells

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Abstract Text: Patchy tissue inflammation is a typical feature of immune-mediated inflammatory diseases (IMIDs) including psoriasis (PsO), Crohn's disease (CD) and ulcerative colitis (UC). Perilesional tissue is not normal, however, with alterations in cellular composition and cytokine profile that apparently resemble an attenuated version of lesional tissue inflammation. Multi-disease comparisons of normal, lesional and perilesional tissue could reveal shared endogenous mechanisms that suppress overt inflammation in these regions, with therapeutic implications. In-house and publicly-available scRNAseq datasets (n=1 and 17, respectively) derived from 356 normal/perilesional and lesional tissues across 18 diseases were integrated and re-annotated to generate a single object of 440,965 cells and derive the largest IMID tissue T cell atlas described to date. The current analysis focussed on comparisons of cellular abundance (logistic regression) and gene programs (fGSEA) between compartments in PsO, CD and UC. Perilesional UC colon exhibited an altered regulatory T (Treg) cell composition compared to healthy colon, with relative expansion of CD25-$_{low}$ Tregs characterised by reduced expression of immunoregulatory genes (IL2RA, FOXP3, TIGIT; OR = 3.70, p< 0.0001), suggesting changes in Treg phenotype could reflect a state of transition to flare in UC. Concerning changes during inflammation, both lesional UC colon and PsO skin displayed depleted CD25-^{high} Treg populations relative to perilesional tissue, with PsO exhibiting comparative expansion of CD25-$_{low}$ Tregs (OR = 1.78, p< 0.0001), suggesting further impairment of immunomodulatory capacity after inflammation has become established. Meanwhile, lesional CD colon featured expanded peripheral helper T (Tph) cells in comparison with perilesional tissue (OR = 2.66, p< 0.0001). Gene set enrichment analysis (GSEA) showed Tph cells in perilesional tissue of all three conditions differ from those of healthy tissue, with upregulated IL-23 signalling and granulocyte-macrophage colony-stimulating factor expression. When compared with lesional tissue, however, the same perilesional Tph cells display attenuated expression of IL-17 and IL-22 in PsO (both p< 0.01), but attenuated IFN-γ expression in CD (p< 0.05). This integrative analysis of an IMID T cell atlas highlights discrete and overlapping pathological features of lesional and perilesional IMID tissue between diseases, particularly suggesting differential Tph/Treg-driven mechanisms by which inflammation may be restrained.

W105. Dynamics of Peripheral Helper T (Tph) Cells Across Multiple Joints and Circulation in Rheumatoid Arthritis

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Abstract Text: Rheumatoid arthritis (RA) is an autoimmune disease characterized by polyarticular inflammation. Oligoclonally expanded $CD4^+$ T cells have been implicated in the pathogenesis of RA, including clonal sharing across different joints. However, the dynamics of these oligoclonally expanded clones across multiple joints and the circulation remain unclear. We performed single-cell RNA/TCR sequencing and cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) of $CD4^+$ T cells in synovium, synovial fluid, and peripheral blood of patients with RA. Unsupervised clustering of 238,788 $CD4^+$ T cells identified 14 clusters, including two distinct subsets of peripheral helper T (Tph) cells: stem-like Tph (S-Tph) and effector Tph (E-Tph) cells, consistent with our recent report (Sci Immunol. 2025). S-Tph cells, which preferentially express CCR7, self-renew and differentiate into E-Tph cells expressing CXCR6, CCR2, and CCR5. To link TCR sequence features with cellular phenotypes, we analyzed whether clonotypes with similar TCR sequences shared gene expression profiles. This analysis revealed a strong association between certain TCR sequence features and Tph-related gene signatures, including CXCL13, MAF, and TOX. Tph cells exhibited distinct TCR sequence features and enriched TCR motifs not observed in other $CD4^+$ T cell subsets, such as regulatory T cells. To explore the dynamics of Tph cells across joints and circulation, we traced TCR clonotypes in synovium and peripheral blood. Synovial E-Tph cells exhibited high clonotype sharing between bilateral joints sampled simultaneously. In addition, substantial clonal overlap was observed in E-Tph cells between the synovium and circulation, whereas it was limited in S-Tph cells. Synovial proliferating cells, marked by high MKI67 expression, shared TCR clonotypes with E-Tph cells in both synovium and blood. Consistent with this, activated circulating E-Tph cells, with the signature of $CXCR5^-PD\text{-}1^{high}HLA\text{-}DR^+$ defined by CITE-seq, showed high clonotype sharing with synovial Tph cells. Circulating E-Tph cells lost CXCR6 expression but retained expression of CCR2 and CCR5, suggesting a potential capacity to migrate to other joints. Collectively, proliferating and activated E-Tph cells circulate across multiple joints and peripheral blood, implying their contribution to polyarthritis. Our study provides insights into the unique TCR features and dynamics of Tph cells underlying the progression of polyarticular rheumatoid arthritis.

W106. PD-L1 on Pancreatic Beta Cells Synergizes with Tregs to Maintain Islet-specific CD8 T Cell Tolerance in Non-autoimmune Settings

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Abstract Text: Despite decades of ongoing research, it has remained difficult to understand the roles of PD-L1 in regulating immunological tolerance. On one hand, PD-L1 deficiency accelerates diabetes in non-obese diabetic (NOD) mice, but by contrast, in non-autoimmune prone settings, PD-L1 loss or blockade does not cause evident autoimmunity, indicating redundancy of PD-L1 in tolerance. However, a fraction of patients receiving checkpoint inhibitor (CPI) blockade can develop rapid-onset and aggressive CPI-induced autoimmune diabetes (CPI-DM) without prior diabetes diagnosis. Also, human with PD-L1-deficiency presented neonatal diabetes without other major autoimmunity. These suggest unrecognized importance of PD-1/PD-L1 pathway in maintaining pancreatic immune tolerance in non-autoimmune prone settings. Here, we used a mouse model called NINJA (iNversion-INduced Joined neoAntigen) to inducibly express neo-self antigens in pancreatic beta cells. Antigen induction led to infiltration of neoantigen-specific endogenous effector $CD8^+$ T cells which eliminated a fraction of beta cells, but did not lead to diabetes due to concomitant loss of T cell functions. Notably, beta cells responded to infiltration with PD-L1 upregulation in an IFNγ- and T cell-dependent manner, and prevented full destruction of islets. Analysis of public ATAC-seq data revealed an open chromatin region at the PD-L1 locus unique to beta cells and tumor lines but not other somatic cell types, indicating beta cell's sensitivity to IFNγ to upregulate PD-L1. Deletion of PD-L1 on beta cells led to increased numbers of CD8 T cells without increased cytotoxicity, along with induction of diabetes by day 9 post antigen-induction. We also observed Treg infiltration, Treg depletion improved effector T cell function even when depletion was limited to days 6-9 post induction. In line with this, combined anti-PD-1 and anti-CTLA-4 treatment led to severe insulitis, with increasing T cell number and function, whereas anti-PD-1 alone only raised T cell numbers. Thus, our data suggests that in non-autoimmune settings, the emerging neo-self antigens in pancreas trigger $IFN\gamma^+$ T cell infiltration and partial beta cell loss. While this simultaneously recruits Tregs and, owing to beta

cells' distinct chromatin accessibility, rapidly induces PD-L1. These coordinated responses restrain islet-reactive CD8 T cells, thereby preserving immune tolerance in pancreas.

W107. Early Detection and Monitoring of Type 1 Diabetes Using a Shared T Cell Receptor Signature

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Abstract Text: Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease characterized by destruction of pancreatic β islet cells. T1D-specific CD4 T cells precede production of T1D-specific autoantibodies, making them attractive early biomarkers of disease. We identified a shared T cell receptor beta (TCRβ) signature that is highly specific to T1D and evaluated its utility for disease classification, early detection, and treatment monitoring. We performed bulk TCRβ sequencing on over 500 $T1D^+$ pediatric cases and over 500 age and HLA-matched (DQ2.5 and DQ8) controls. We discovered a set of shared TCRβ sequences that are significantly enriched in individuals with T1D. We demonstrated that this T cell signature robustly discriminates cases from controls in a holdout set from multiple independent and geographically diverse cohorts, and that clonal expansion of these shared T cells can be detected prior to detection of autoantibodies, sometimes years before seroconversion. To assess the clinical relevance of this signature in therapeutic contexts, we analyzed TCRβ repertoires from participants enrolled in T1D immunotherapy clinical trials, both before and after treatment. Notably, a reduction in the T cell signature following treatment was associated with improved clinical outcomes, including improved HbA1c and C-peptide AUC. Together, these findings demonstrate that TCRβ sequencing captures disease-relevant T cell activity in T1D. We propose that TCRβ sequencing represents a valuable tool for early disease detection and for monitoring treatment response in T1D clinical trials.

W108. Discovery of Autoimmune T Cell Antigens Through Transsynaptic Lymphocyte Adhesion Biosensing

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Abstract Text: Defining the peptide–MHC targets of disease-expanded T cells is central to understanding autoimmune pathogenesis, yet remains constrained by HLA diversity, the breadth of self-antigens, and limitations in discovery methods. We developed a cell-based antigen discovery platform that converts integrin-mediated mechanical forces at the immunologic synapse into a selectable genetic signal, with barcoding enabling pooled tracking of peptide and MHC identity. This framework enables direct functional interrogation of individual T cell clones expanded at sites of disease, including tissue and cerebrospinal fluid (CSF). Applied to a murine model of autoimmune myocarditis, this approach resolved the antigen specificities of disease-expanded CD4 and CD8 T cell clones, identifying both known and novel epitopes derived from Myh6, a canonical cardiac autoantigen, as well as peptides from previously unimplicated proteins. We identified multiple instances of TCR cross-reactivity to self-antigens, providing new insight into mechanisms of self-antigen recognition. We further applied this strategy to expanded human CD4 T cell clones isolated from the CSF of a patient with ROHHAD (rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysregulation), identifying novel HLA-DQ–restricted peptides derived from a central nervous system autoantigen. Notably, these peptide–MHC targets overlapped with disease-associated autoantibodies, supporting antigen-linked B cell–T cell crosstalk in CNS autoimmunity. Together, these findings establish a functional, unbiased framework for T cell antigen discovery grounded in disease-expanded clones, while uncovering cross-reactive and shared antigenic drivers of autoimmune pathology.

W109. Immune Checkpoint Activation Targeting PD-1H (VISTA) Reinforces Human T Cell Tolerance in Autoimmunity

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Abstract Text: Immune checkpoints regulate immune tolerance, and while immune checkpoint inhibitors enhance anti-tumor T cell immunity, autoimmune diseases arise from unchecked immune activation and may benefit from the opposite strategy. Immune checkpoint activators (ICAs) offer a promising approach to restore tolerance without broad immunosuppression. PD-1H (also known as VISTA or VSIR) is an inhibitory checkpoint expressed on T cells and myeloid cells, with emerging evidence implicating its role in autoimmune regulation. Here, we investigate PD-1H activation as a strategy to suppress pathogenic human T Cell responses and reinforce immune balance in autoimmunity. We analyzed public RNA-seq datasets from lupus nephritis patients and healthy controls and performed multiparameter flow cytometry of healthy and lupus PBMCs to assess PD-1H expression and clinical associations. An *in vitro* T Cell exhaustion model with CRISPR–Cas9–mediated PD-1H disruption was used to define PD-1H's role in regulating human T cell exhaustion. A lead human PD-1H agonistic monoclonal antibody was identified and evaluated *in vitro* and *in vivo* using human T cell exhaustion assays and patient-derived xenograft (PDX) models. PD-1H was preferentially upregulated on antigen-experienced $CD4^+$ and $CD8^+$ T cells and sustained under chronic stimulation. PD-1H deficiency enhanced T Cell proliferation, survival, and inflammatory cytokine production, accompanied by reduced $PD\text{-}1^+TIM\text{-}3^+$ exhausted subsets, indicating a role in restraining prolonged immune activation. Analysis of the AMP lupus nephritis dataset revealed high PD-1H expression on renal-infiltrating effector T cells, exceeding other inhibitory checkpoints. Flow cytometry confirmed $PD\text{-}1H^+$ T cells in lupus PBMCs, with expression correlating positively with clinical disease activity. PD-1H immune checkpoint activation suppressed early T Cell activation and inflammatory cytokine production and, under chronic stimulation, reinforced an exhaustion-like program marked by increased PD-1, TIM-3, and TOX expression. In humanized GvHD models, PD-1H activation reduced disease severity and prolonged survival. In lupus PDX models, PD-1H activation ameliorated renal pathology, reduced proteinuria, and selectively depleted pathogenic T Cell populations while enriching exhausted subsets. Together, these findings identify PD-1H as a key checkpoint regulating chronic human T Cell responses and provide preclinical proof-of-concept that immune checkpoint activation can restore immune tolerance by enforcing exhaustion of pathogenic T cells, supporting PD-1H agonism as a promising therapeutic strategy for autoimmune disease.

W110. Infiltrating Monocytes in Lupus Nephritis Acquire Disease-associated Signatures Linked to Glomerular Factors and Modulate Mesangial Repair Pathways

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Abstract Text: Lupus nephritis (LN) is the most common and severe organ manifestation of systemic lupus erythematosus (SLE), affecting up to 60% of patients and driving SLE-related morbidity and mortality through progressive kidney injury and end-stage kidney disease. We recently identified a population of infiltrating, disease-associated monocytes that strongly correlates with the histologic activity index, as well as a distinct population of putative resident macrophages that correlates with the chronicity index, a measure of irreversible damage; neither population was detectable in healthy kidneys. We refer to these populations collectively as disease-associated monocyte/macrophage (DMacs). Here, we aimed to explore the role of DMacs by investigating their interactions with renal stromal cells to uncover mechanisms contributing to kidney damage in LN. We first mapped the positions of DMacs in active LN kidneys using Xenium spatial transcriptomics with myeloid-focused probes. Infiltrating monocytes densely accumulated within nephritic glomeruli, whereas resident macrophages localized predominantly

to the tubulointerstitium. To enable functional studies of DMacs, we sought to infer their origins and identify tissue factors associated with their in situ differentiation. Analysis of single-cell datasets from kidney biopsies of patients with active LN and healthy controls suggested that DMacs likely derive from infiltrating monocytes and upregulate a conserved injury-associated gene program characterized by canonical markers such as TREM2, APOE, SPP1, GPNMB, and others, consistent with macrophage responses across diverse injured tissues. To model this process, we stimulated peripheral $CD14^+$ monocytes *in vitro* with factors associated with injured glomeruli—apoptotic and necrotic renal debris, TGF-β, and M-CSF—identified in our analyses. Monocytes upregulated the injury-associated gene program and shifted toward a DMac-like transcriptional phenotype. To assess functional consequences, we performed scratch-wound assays using primary glomerular mesangial cells treated with media conditioned by induced DMacs. Compared to unstimulated controls, DMac-conditioned media accelerated wound-closure kinetics and improved final wound coverage, suggesting that DMacs can modulate mesangial repair responses. Collectively, our findings support a model in which infiltrating monocytes in LN adopt injury-associated DMac states in response to glomerular niche signals and participate in shaping glomerular repair processes, highlighting these cells as a potential therapeutic target.

W111. B Cell–intrinsic 3BP2 Signaling Drives the Pathogenesis of Systemic Lupus Erythematosus

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Abstract Text: [Background] 3BP2 is an adapter protein that mediates tyrosine kinase signaling and plays an important role in osteoclastogenesis. While we have revealed that 3BP2 regulates TLR2-related intracellular signaling in myeloid cells, its role in lymphocyte signaling and autoimmune diseases, including systemic lupus erythematosus (SLE), remains unknown. [Objectives] To verify the role of 3BP2 in the pathogenesis of SLE and clarify its potential as a therapeutic target for SLE. [Methods] We analyzed the expression levels of 3BP2 in immune cells from patients with SLE and lupus-prone mice. We generated 3BP2 knockout (KO) mice in a lupus-prone background (B6/lpr CD72 KO). Histological evaluation of various organs and analysis of urine albumin and serum autoantibodies were performed. Immune cell subsets were analyzed by Flow cytometry (FCM). Single-cell RNA-sequence (scRNA-seq) was performed using peripheral blood mononuclear cells from the spleen of these mice. Western blot and quantitative PCR were performed to analyze the effect of 3BP2 deficiency on cell signaling and cytokine production. [Results] We found that the expression levels of 3BP2 are increased in some types of immune cells, including B cells from both patients with SLE and lupus-prone mice. Of note, the deletion of 3BP2 led to a marked reduction of the urine Alb/Cr ratio in lupus-prone mice (B6/lpr CD72KO mice). Inflammatory cell infiltration into the lung, liver, and kidney was also improved in lupus-background 3BP2KO mice. By FCM, we found that some cell-activating markers are upregulated in lupus-prone mice and improved by deletion of 3BP2. scRNA-seq analysis identified that Age or autoimmune-associated B cells (ABCs) were decreased and some signal pathway-associated genes were altered by the deletion of 3BP2. *In vitro* experiments revealed that 3BP2 regulates TLR7/9 downstream signaling in B cells. [Conclusions] These findings identify 3BP2 as a key regulator of B cell–intrinsic innate-like signaling pathways that drive SLE pathogenesis and highlight 3BP2 as a potential therapeutic target in SLE.

W112. CD1a-targeted PD-1 Agonist for Localized Immunosuppression as a Therapeutic Approach in Atopic Dermatitis

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Abstract Text: T cells play a central role in the pathogenesis of atopic dermatitis (AD). In the context of skin barrier defects, they respond to environmental allergens and orchestrate the ensuing inflammatory response. Programmed

cell death protein-1 (PD-1) is a key immune checkpoint that restrains T cell activity; PD-1 signalling raises the threshold for T cell activation, limits effector responses, promotes resolution of inflammation, and contributes to T cell tolerance. We have developed a skin targeted PD-1 agonist as a novel strategy to potentially treat inflammatory skin diseases. These cell-bridging bispecific molecules combine: (i) a high affinity VHH targeting domain specific for CD1a, an HLA related molecule highly expressed on skin antigen presenting cells (APCs), and (ii) a PD-1 agonist VHH to modulate T cell activity at the immune synapse. In coculture assays using $CD1a^+$ or $CD1a^-$ APCs, together with nontargeted PD-1 agonist control bispecifics, we show that CD1a directed PD-1 agonists suppress T cell activation only when target-bound. The CD1a targeted PD-1 bispecific does not compete with PD-L1 or PD-L2 for PD-1 binding. Coculture studies using monocyte derived Langerhans cells (moLCs), which express both PD-1 ligands, demonstrate that the bispecific acts additively with endogenous PD-1 ligands to enhance T cell suppression. The bispecific potently inhibits MoLC-stimulated Jurkat NFAT reporter activity and house dust mite (HDM)–stimulated IL-13 release in autologous MoLC–T cell assays. Target engagement studies in ex-vivo human skin explants show specific binding of fluorescently labelled bispecific molecules to $CD1a^+$ APCs in both healthy and AD skin. Furthermore, quantitative immunohistochemistry and cytokine array analyses demonstrate that the bispecific suppresses T cell proliferation and activation in AD lesional and perilesional skin. Collectively, these data support CD1a targeted PD-1 agonists as a promising approach to achieve localized inhibition of inflammatory T cells in the skin while avoiding systemic immunosuppression.

W113. Tissue-specific Diversity and Depletion Sensitivity of B Cell Subsets in the NOD Mouse Model of Type 1 Diabetes

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Abstract Text: Type 1 Diabetes (T1D) is a T cell–mediated autoimmune disease characterized by cytotoxic destruction of pancreatic β cells; however, B cells play critical roles in disease initiation and progression. Clinical studies using B cell depleting antibodies, such as rituximab, demonstrate delayed disease progression, yet emerging strategies, including antigen-specific depletion and chimeric antigen receptor (CAR) T cell therapy, remain under-explored in autoimmune diabetes. Given the phenotypic and functional heterogeneity of B cells, defining which subsets expand or contract before and during disease is essential for understanding their pathogenic roles. Moreover, determining how different depletion strategies affect these subsets may reveal mechanisms of protection and inform therapeutic design. To address this, non-obese diabetic (NOD) mice aged 3–5 weeks, 10–12 weeks, and at disease onset were profiled by flow cytometry to characterize B cell subsets across bone marrow, spleen, blood, pancreatic and brachial lymph nodes, pancreatic islets, and peritoneal fluid. Subset distributions were compared across tissues and disease stages. Additional cohorts were treated with anti-CD20 antibodies or CD19-directed CAR T cells to evaluate depletion efficiency and residual B cell phenotypes across tissues. Comparative analysis revealed age- and tissue-specific shifts in B cell composition. In pancreatic and brachial lymph nodes, older mice exhibited increased frequencies of follicular B cells, whereas younger mice showed more germinal center and marginal zone B cells. The most pronounced differences were observed within pancreatic islets: young mice harbored follicular and developing/immature B cells, while older mice showed marked enrichment of B1 cells, particularly the B1b subset. Preliminary depletion studies demonstrated effective overall B cell reduction one month after treatment. CAR T cell therapy achieved broad depletion across all B cell subsets, while anti-CD20 treatment failed to eliminate bone marrow B cells and tissue-resident B1 populations, including those in the peritoneal cavity and pancreas. These findings demonstrate dynamic, age-dependent changes in B cell subsets during T1D progression and highlight fundamental differences in depletion breadth and depth between antibody- and CAR T–based therapies. Ongoing studies will assess whether broader B cell depletion confers enhanced protection from T1D, providing insight into pathogenic contributions of distinct B cell subsets.

W114. Positive Clinical Translation of a Juvenile NHP Study for Evaluating SAB-142, a Multi-specific T Cell Targeting T1D Therapy, in Humans

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Abstract Text: Background and Aims SAB-142 is a fully human, multi-specific, targeted Anti-Thymocyte Globulin (hATG) developed for delaying the onset and progression of T1D with the capability for safe and effective redosing. A 9-month, two-dose chronic GLP toxicology study in 30 juvenile cynomolgus monkeys was conducted to evaluate the toxicity, toxicokinetic, and immunologic effects of SAB-142. Pharmacological outcomes of this study were compared to outcomes from a first-in-human Phase 1 clinical study of SAB-142 to assess clinical translatability. Methods In this positively predictive GLP toxicology study, three dose levels of SAB-142 were administered at study initiation and re-dosed at ~6 months. This study was designed to mimic the regimen of every 6-months dosing and provided up to a 20-fold safety margin of target dose levels in a Phase 1 and an ongoing Phase 2b clinical trial. Results The 9-month chronic GLP toxicology study demonstrated that SAB-142 was safe and well tolerated in juvenile cynomolgus monkeys. Consistent with its expected pharmacological activity, SAB-142 induced a transient, dose-proportional reduction in total peripheral lymphocyte counts following an initial induction dose. This transient lymphocyte response was reproduced following a maintenance re-dose at ~6 months. Reduction of lymphocytes was short-lived and total lymphocytes returned to baseline after a few days following treatment of SAB-142, suggesting a mechanism of lymphocyte margination not lymphodepletion. This effect was observed in both cynomolgus monkeys and in human subjects evaluated in the Phase 1 study. Conclusions The positive translational value of this 9-month, two-dose chronic GLP toxicology study was supported by Phase 1clinical data, with both studies suggesting lymphocyte margination not sustained lymphodepletion across all tested dose levels of SAB-142. In the Phase 1 clinical study, peripheral lymphocyte counts returned to baseline by Day 7, in both healthy volunteers and patients with stable T1D, confirming the clinical translation of the transient pharmacodynamic effect observed in this preclinical toxicology study in cynomolgus monkeys.

W115. Discovery and Engineering of Novel IgM Proteases for the Treatment of Autoimmune and Inflammatory Diseases

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Abstract Text: IgM is a multifunctional immunoglobin that acts as the first line of defense against systemic pathogens. IgM's multimeric structure, together with its complement binding ability, makes IgM a highly potent inflammation driver. The presence of autoreactive IgM antibodies causing complement dependent damage has been described in inflammatory and autoimmune conditions such as Cold Agglutinin Disease (CAD) and Multifocal Motor Neuropathy (MMN) . For this reason, the development of IgM autoantibodies degrading therapy represents an attractive approach. Employing Seismic's IMPACT platform and evolutionary screening, we identified several novel and selective IgM proteases. Discovered proteases cleave soluble IgM in plasma and the IgM BCR in B cells from healthy individuals. CAD is an autoimmune hemolytic anemia, caused by IgM autoantibodies that bind to red blood cells (RBC), causing agglutination and activate complement cascade resulting in RBC death. Seismic's IgM protease cleaves RBC bound IgM, preventing agglutination in whole blood from CAD donors, an unmet need not targeted by current approved treatment. In addition, IgM cleavage reduced cold-induced complement deposition on RBC from CAD patients. Further engineering and optimization with our IMPACT Platform for the novel proteases is currently under way.

W116. Uncovering a Critical Role for MALT1 in TNF-driven Arthritis and Ileitis

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Abstract Text: Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is a key player in the activation of immune and non-immune cells by functioning both as a scaffold protein as well as a paracaspase. This is reflected by MALT1's fundamental role in the pathogenesis of auto-immune mouse models for rheumatoid arthritis and multiple sclerosis. In contrast, TNF signaling is widely considered to operate independently of MALT1, leaving the contribution of MALT1 to cytokine-driven inflammatory disease unresolved. Here, we examined the role of MALT1 in a TNF-driven mouse model (TnfΔARE) that recapitulates key features of human spondyloarthritis, including chronic arthritis and ileitis. Genetic deletion of Malt1 in TnfΔARE mice resulted in robust protection from both joint and intestinal inflammation, as demonstrated by clinical scoring and histopathological analysis. This protection was accompanied by markedly reduced expression of inflammatory mediators at the respective sites of inflammation, despite preserved Tnf expression. Strikingly, serum TNF and soluble TNF receptor (sTNFR) levels remained equally elevated in MALT1-deficient and control TnfΔARE mice, indicating that disease attenuation occurred despite sustained systemic TNF signaling. These findings uncover an unexpected requirement for MALT1 in TNF-driven pathology. To dissect the cellular basis of disease protection, we performed complete bone marrow transfer experiments. These demonstrated that MALT1-deficiency in the hematopoietic compartment was sufficient to confer robust protection from ileitis. In contrast, protection from arthritis was less clear-cut, suggesting contributions from both hematopoietic and non-hematopoietic compartments. Consistent with this, myeloid-restricted deletion of MALT1 using LysM-Cre failed to recapitulate the protective phenotype, and MALT1-deletion in synovial fibroblasts did not alter intrinsic inflammatory responsiveness *ex vivo*. Together, these findings indicate that TNF-driven arthritis arises from cooperative MALT1-dependent interactions between hematopoietic and stromal compartments rather than from a single disease-driving cell type. Overall, our data identify MALT1 as a previously unrecognized and context-dependent modulator of TNF-driven inflammatory disease, acting through distinct cellular mechanisms in intestinal and joint tissues. These findings challenge the prevailing view of TNF signaling independence from MALT1 and highlight cell type- and tissue-specific pathways that may be exploited for therapeutic intervention in spondyloarthritis.

W117. Immunity to Influenza B and Cross-reactivity to Pandemrix/pH1N1 in Narcolepsy

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Abstract Text: 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 NT1onset. 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. With thorough analysis of their sequences, we found a B/Victoria PB1 epitope that is homologous to Pandemrix/pH1N1 PB1 segment with only one amino acid difference. Using tetramer DQ0602, we detected reactive $CD4^+$ T cells to this epitope in two cohort studies using different subjects. PB1 cells from the first cohort were sorted out for single-cell RNA sequencing and over 6,000 cells were recovered. Over 90% were effector memory T cells, over 86% were from multiple NT1 patients, and more than 35 clones were enriched at least 20 times, including 2 with over 500 times. These clones were activated by both

peptides from B/Victoria and Pandemrix/pH1N1 under NFAT and NF-κB pathways but not AP-1, suggesting molecular mimicry between influenza A and B through PB1 epitope. In addition, we found TCR clones from baseline PBMCs were activated by Pandemrix/pH1N1. Conclusion: Our research showed influenza B plays a role in NT1 pathogenesis and cross-reactivity to PB1 between B/Victoria and Pandemrix/pH1N1.

W118. Regulation of DN2 and ABC B Cells by IL-12 and IFNγ During Systemic Lupus Erythematosus and Sjogren's Disease

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Abstract Text: Systemic lupus erythematosus (SLE) and Sjögren's disease (SjD) are chronic autoimmune disorders that, despite distinct clinical presentations, share a common feature: persistent overactivation of B cells. Recent evidence indicates that abnormal B cell responses occurring outside the typical germinal centers (GC) of secondary lymphoid structures—termed extrafollicular (EF) responses—play a central role in the onset and progression of these diseases. In the chronically inflamed environment of SLE patients, EF responses generate pathogenic B cell subsets, including double-negative 2 (DN2) B cells in humans (termed age-associated B cells (ABCs) in mice) and plasmablasts (antibody secreting cells). In some instances, exuberant EF responses coincide with suppressed GC. We have identified the cytokine IL-12 as a key driver that enhances EF responses while coordinately suppressing GC. IL-12 acts directly on B cells to induce production of interferon-gamma (IFNγ), and together these signals promote B cell differentiation. The clinical relevance of the IL-12 signaling pathway in SLE and SjD is underscored by genetic and clinical observations: variants in IL-12 signaling rank among the strongest genetic risk factors for both SLE and SjD (GWAS), and elevated IL-12 levels correlate with SLE disease flares. Despite this, the mechanisms by which IL-12 promotes B cell differentiation remain poorly understood. Our preliminary data demonstrate that IL-12 can drive the formation of DN2-like cells and plasmablasts from human B cells, and ABC and plasmablasts from mouse B cells. While the role of IFNγ in shaping these cell populations is established, IL-12's direct effects remain unexplored. Our studies aim to define how IL-12 and IFNγ, individually and in combination, regulate the differentiation of DN2 and ABC populations in human SLE and SjD, as well as in murine lupus models. We also aim to understand how these pathogenic B cell subsets remain responsive to further IL-12 and IFNγ stimulation, which could establish feed-forward loops that perpetuate disease. These findings will clarify the role of IL-12 in driving autoimmune B cell responses and may identify new therapeutic targets for patients with SLE and/or Sjögren's disease.

W119. The Oxygen Sensor PHD1 Is a Critical Regulator of Arthritis Development and Joint Inflammation

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Abstract Text: Objectives: Rheumatoid arthritis (RA) is a systemic autoimmune disease resulting in chronic inflammation and joint damage. Synovial joints exist under relatively low oxygen tension, and further reductions in oxygen availability during RA create a hypoxic cellular environment that contributes to joint pathogenesis. In this cellular response, hypoxia-inducible factor (HIF) plays an important role under the regulation of oxygen-sensing prolyl hydroxylases (PHD1–3). These PHDs also display HIF-independent functions involving the regulation of NF-kB, and might impact the inflammatory response in RA. However, the exact contribution of PHDs to RA development remains poorly understood. Methods and results: We first investigated the effect of environmental hypoxia on disease progression in a passive transfer arthritis model (collagen antibody-induced arthritis). Mice housed in hypoxia (10% O2) versus normoxia (21% O2) presented lower clinical arthritis scores and reduced inflammation and damage in the knee joint. To investigate the role of the individual PHD oxygen sensors, we then subjected mice with germline deficiencies of Phd1, Phd2 or Phd3 to the same arthritis model. Whilst $Phd2^{+/-}$ and $Phd3^{-/-}$ mice showed

similar clinical symptoms compared to controls, Phd1 deletion protected against arthritis symptoms based on clinical scoring and histopathology. By then probing a human synovial single-cell RNA-seq dataset, we could show that macrophages are among the main cell types with a robust hypoxic transcriptional signature in RA patients. Since macrophages are also key players in joint inflammation, we next questioned whether macrophages are involved in the PHD1-mediated control of arthritis. Myeloid-specific deletion of Phd1 indeed resulted in similar protection against arthritis compared to whole-body Phd1 deficiency. This does not appear to be dependent on an M1-to-M2 macrophage polarization, but rather a hypoxia- and PHD1-driven joint infiltration of macrophages. Indeed, a reduced expression of chemoattractants (e.g., CCL2, CCL3 and CCL4) was observed in both synovium from diseased mice and cultured human macrophages under hypoxic conditions. Conclusion: Our data indicate that the oxygen sensor PHD1 is a critical regulator of arthritis development and a potential target for the treatment of arthritis. We show that these protective effects are macrophage-driven and the resultant of reduced macrophage infiltration in the inflamed joint.

W120. Single Cell Analysis of Cutaneous Lupus and Scleroderma Skin Biopsies Highlights Shared and Disease-Specific Patterns

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Abstract Text: In this study, we generated a large-scale skin scRNA-seq dataset comprising over 250,000 cells of disease affected and unaffected biopsies (n=82 total) from individuals with Cutaneous Lupus (CL, n=17) and Scleroderma (SSc, n=16), along with healthy controls (HC, n=10). Although CL and SSc are distinct in presentation and pathology, we hypothesize that studying them together – given they both manifest in skin – may reveal shared biological states that contribute to skin autoimmune activation. Despite differences in cellular composition for affected CL (increased immune) and SSc (increased Endothelial) versus HC, we identified several shared patterns. Together, differential gene expression and Spectra factor analysis revealed broad upregulation of Interferon across cell types in CL but also in a subset of Endothelial Cells in SSc. We additionally see shared increases in Epithelial Mesenchymal Transition and Extracellular Matrix Composition across non-immune cell types, and shared changes in Keratinocytes related to decreased differentiation and increased senescence. Cell-cell communication analysis revealed a shared increase in MK signaling from Endothelial Cells and Fibroblasts. Finally, we see shared abundance shifts in affected versus unaffected tissue, both globally -- driven by decreased Keratinocytes -- and in Immune cells -- driven by decreased tissue resident T Effector Memory and Langerhans cells and increased activated T cells. Our work demonstrates the value of cross-autoimmune disease analysis in tissue for uncovering both disease-specific and shared states in CL and SSc. Extending this framework to additional skin autoimmune conditions has the potential to refine our understanding of common immune mechanisms and reveal therapeutic targets.

W121. Senescent Synovial Fibroblasts Orchestrate the Pathogenic Niche and Drive Disease Flares in Rheumatoid Arthritis

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Abstract Text: Biologic disease-modifying anti-rheumatic drugs (b-DMARDs) have revolutionized the treatment of Rheumatoid Arthritis (RA); however, approximately 40% of patients remain refractory to current therapies. Recent large-scale clinical trials and integrated single-cell transcriptomic analyses have revealed that this multi-drug

resistance is frequently driven by a "fibroblast-rich" synovial pathotype. Consequently, elucidating the mechanisms of fibroblast-mediated inflammation is an urgent priority. Accumulating evidence points to the critical role of cellular senescence, a state characterized by irreversible cell cycle arrest that triggers chronic inflammation through the senescence-associated secretory phenotype (SASP). We hypothesized that the accumulation of senescent fibroblasts establishes a "senescent niche", which contributes to sustained inflammation and disease flares. First, we performed transcriptomic profiling across multiple independent datasets, which consistently revealed a significant enrichment of senescence signatures in RA synovium compared to osteoarthritis and healthy controls. Next, to characterize the cellular heterogeneity of RA synovium, single-cell RNA sequencing identified sublining fibroblasts as the predominant senescent population, exhibiting high expression of SASP factors. Ligand-receptor analysis highlighted these cells as communication hubs, orchestrating innate immune cascade activation and driving stromal expansion via specific mitogenic signaling. Immunohistochemical analysis of RA synovium visualized a spatial "senescent niche" where highly proliferative fibroblasts cluster around senescent fibroblasts. To elucidate the role of senescence in clinical flares, we utilized a methylated bovine serum albumin (mBSA)-induced arthritis model. We observed that senescent fibroblasts accumulated after the initial induction, and upon re-challenge, mice exhibited exacerbated inflammation. Importantly, in a transgenic mouse model, the selective elimination of p16-positive senescent cells significantly attenuated inflammation-related gene expression, including CCL and CXCL chemokines, and ameliorated arthritis severity during re-challenge. Our findings demonstrate that senescent fibroblasts contribute to synovial inflammation and actively orchestrate disease flares in RA. These findings suggest that targeting senescent cells offers a transformative therapeutic strategy to overcome treatment resistance and achieve functional recovery in refractory RA patients.

W122. Monocyte Phenotypes in Blood Predict Response to TNF Inhibitors in Rheumatoid Arthritis

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Abstract Text: TNF inhibitors (TNFi) are the most common biologic DMARD used to treat RA yet 25-40% of patients who start a TNFi do not obtain even 20% improvement. Thus, there is a great need to understand the biology driving clinical response to TNFi and to identify biomarkers to guide the choice of treatment. We hypothesized that detailed cellular profiling on blood samples from RA patients can identify immune cell features that are associated with TNFi treatment response. We utilized PBMC collected as part of the TARGET RCT (Solomon et al., Ann Rheum Dis, 2023), which randomized RA patients with an inadequate response to methotrexate to either addition of TNFi (adalimumab or etanercept; n=51) or 'triple' therapy (sulfasalazine and hydroxychloroquine; n = 47). Patients were 72% female with a median age of 60. Treatment response was determined by DAS28 EULAR criteria at 24 weeks. We performed scRNA sequencing on PBMC from baseline and week 24. TNFi induced substantial changes in phenotypes of every major immune cell lineage. Our analyses highlighted shifts in phenotypes of $CD14^{+}CD16^{-}$ monocytes comparing baseline and post TNFi samples. These changes were observed in TNFi treated patients and not in the triple therapy group. We compared immune profiles of baseline samples from responders and non-responders to identify cellular features in baseline samples associated with subsequent response to TNFi. Abundance of the monocyte phenotype altered by TNFi in longitudinal analyses differed significantly between TNFi responders and non-responders. These TNFi response-associated monocytes express inflammatory factors including IL1, CCL2, and CXCL8 all of which are more highly expressed by TNFi responders. Transcriptomic mapping of myeloid cells in our analysis to myeloid cells from RA synovial tissue revealed similarities of the TNFi response-associated monocytes to inflammatory macrophages in RA synovial tissue. Analysis of differentially expressed genes encoding surface proteins yielded a candidate list of surface factors, which we validated by flow cytometry. Cytometry quantification of these markers successfully reproduced the discrimination of TNFi responders and non-responders in a subset of TARGET patients. These monocytes can be quantified in blood using cytometry, which may facilitate translation of a predictive biomarker test to clinical application.

W123. Knockdown of the Long Isoform of the Prolactin Receptor Selectively Targets Pathogenic Immune Cells in Systemic Lupus Erythematosus and Averts Glomerular Pathology

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Abstract Text: Rationale: Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by chronic immune activation, sustained inflammation, and progressive damage to multiple organ systems. Although elevated circulating prolactin (PRL) levels have been associated with increased disease activity in SLE, the mechanisms by which PRL signaling contributes to disease pathogenesis remain poorly defined. PRL binds and signals through the prolactin receptor (PRLR), a type I cytokine receptor. The PRLR has antagonistic long (LF) and short (SF) splice variants: in contrast to the SF, the LF is pro-proliferative and anti-apoptotic. In this study, we determined the effects of aberrant PRLR splicing on immune cell pathology and organ damage in SLE. Methods: We analyzed the expression of PRLR splice variants in immune cells in preclinical models of human and mouse SLE. To determine whether aberrant PRLR isoform expression drives SLE, we used a splice-modulating oligomer (SMO) to selectively knockdown the LFPRLR *ex vivo* in peripheral blood mononuclear cells (PBMCs) of patients with SLE, and *in vivo* in lupus-prone mice. We measured immune cell composition, activation states, and transcriptional programs using high-dimensional flow cytometry and single-cell RNA-sequencing. We evaluated the impact of long-term, *in vivo* LFPRLR knockdown on glomerular pathology. Results: Compared to their healthy counterparts, PBMCs from patients with SLE had aberrant PRLR splicing, characterized by a significantly increased ratio of long to short PRLR splice variants. Selective LFPRLR knockdown reduced immune cell-expression of type I interferon signaling and response genes known to drive SLE. LFPRLR knockdown also diminished B cell immunoglobulins with features of autoreactivity and significantly attenuated glomerular pathology in lupus-prone mice. At a cellular level, pathogenic B cell- and other immune subsets (monocyte, NK, and T cell subsets) that promote B-cell activation and survival, were selectively reduced, without negative impact on healthy donor counterparts. Conclusions: Aberrant expression of LFPRLR drives pathogenic immune cell phenotypes and organ damage in SLE. Targeted modulation of PRLR splicing using LFPRLR SMO mitigates key molecular, cellular, and tissue-level features of disease without broadly suppressing healthy immune cells. Being a mechanistic contributor to SLE pathogenesis, LFPRLR represents a promising therapeutic target that merits further investigation.

W124. Lupus Nexus: A Registry, Biorepository, and Data Exchange Platform to Foster Global Collaborations, Accelerate Research, and Advance Precision Medicine

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Abstract Text: Systemic lupus erythematosus (SLE) remains a disease of high unmet medical need. 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. The Lupus Nexus (LNx) is a lupus registry, biorepository, and data exchange platform that addresses this need. 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. The clinical data includes clinician-reported outcomes (SLEDAI-Flare Index, SLICC/ACR Damage Index, neuropsychiatric SLE, SLEDAI-2K, PGA-VAS), patient-reported outcomes (sociodemographic, health habits, SLAQ, PROMIS, Lupus Quality of Life), full medical history (including familial autoimmune, serological, medications, and vaccination history), social history and determinants of health, and environmental exposures, among others. Linked biospecimens include PBMCs, DNA, RNA, urine, plasma, serum, saliva, stool, and tissue. To date, 635 participants have been enrolled. The registry is reflective of the population affected by lupus with 36% Black, 39% White, 12% Asian/Pacific Islander, and 16% Hispanic patients. Over 12,000 biosamples have been collected. Targeted genetic, transcriptomic, proteomic, quantitative auto-antibody measurements, microbiome metagenomics, and medication level assessments have been generated for 200 participants, with more biosamples in process, to support preliminary research efforts and further utilization of LNx resources. LNx is the first of its kind collaborative, patient-centric, lupus research platform poised to become the leading source of prospective, longitudinally well-phenotyped, comprehensive data with linked biospecimens and raw biomarker data available to the entire research community. This initiative aims to accelerate precision medicine in lupus, driving significant advances in diagnosis and treatment. The LRA acknowledges the many experts that have contributed to its creation, especially those individuals living with lupus and their care partners. www.lupusnexus.org

W125. Genetic Predisposition and Viral Infection Impact the Development of Autoantibodies in Murine Systemic Lupus Erythematosus (SLE)

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Abstract Text: Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by the generation of autoantibodies to numerous nuclear and intracellular antigens. SLE results from a combination of environmental factors and genetic predisposition, although much of the etiology of SLE remains unknown. Viral infection has been associated with exacerbation of disease, but there is little known about whether virus alone can induce SLE. We hypothesize that viral infection plays a key role in the initiation and progression of SLE. We investigated whether infection of mice with lymphocytic choriomeningitis virus Armstrong (LCMV-Arm) impacts the production of autoantibodies in a model of murine SLE induced in nonautoimmune C57BL/6 mice by immunization with β2-glycoprotein I and lipopolysaccharide. We found that infection with LCMV-Arm 6 days following the induction of SLE resulted in a sustained increase in hallmark SLE autoantibodies (including anti-DNA, anti-Ro, anti-La, anti-Sm and anti-Sm/RNP), compared to uninfected immunized mice. We then asked whether LCMV-Arm infection has the potential to induce SLE autoantibodies in mice with a genetically autoimmune-prone background. We found that LCMV-Arm infection resulted in elevated anti-DNA autoantibodies ~3 weeks following infection in age- and MHC II-matched autoimmune-prone (New Zealand Black [NZB]) and nonautoimmune (BALB/c) mice. However, there was a subsequent decrease in anti-DNA levels in both strains. Anti-DNA levels in infected BALB/c mice remained elevated compared to uninfected BALB/c mice, but gradually decreased over time. In contrast, anti-DNA levels in infected NZB mice tended to be lower than those in uninfected NZB mice, but rose to similar or higher levels ~16 weeks after infection. Other SLE autoantibodies, including anti-Ro, anti-La, anti-Sm, and anti-Sm/RNP, were not elevated in either LCMV-Arm infected or uninfected NZB and BALB/c mice. We are also evaluating the impact of LCMV-Arm infection on a murine strain (MRL/MpJ) that is more overtly autoimmune than NZB, together with a nonautoimmune (C3H/He) MHC-II matched control strain. Our data suggest that LCMV-Arm infection impacts the initiation and development of SLE autoantibodies differently in autoimmune-prone strains compared to nonautoimmune strains of mice.

W126. The IL-2/IL-2R Signaling Axis Between Tfh and Regulatory CD4 Subsets Is Dysregulated in Islet Autoreactive T Cells of Individuals with T1D

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Abstract Text: Rare autoantigen specific T cells can be enumerated in healthy controls and autoimmune disease, but characterizing underlying mechanisms that alter their function remains a challenge. Understanding defects in autoreactive T cells in type 1 diabetes (T1D) is particularly important as antigen-specific (A-S) therapies are being trialed to induce tolerance. Here, we explore the characteristics of preproinsulin (PPI)-specific CD4 T cells in early onset T1D participants in the TrialNet TOPPLE study prior to treatment with a PPI plasmid therapy. We used an activation induced marker (AIM) assay wherein PBMC are stimulated overnight with PPI or viral peptide libraries before staining for activation and other phenotypic markers. Cells expressing multiple activation markers by flow cytometry were considered A-S ($CD154^+CD69^+$ T conventional; Tconv, $CD137^+CD25^+$ Treg) and further characterized. Overall, we found a discordance between PPI-specific and viral-specific T cell phenotypes. Specifically, the frequency of $CD25^+$ (high affinity IL-2R) and $CXCR5^+$ (T follicular helper, TfH) viral-specific CD4 memory cells positively correlated with these same populations in global CD4 conventional (TConv) cells (r=0.602 and r=0.453, respectively), but PPI-specific cells did not. Additionally, viral-specific Tregs and Tfh were positively correlated (r=0.604), but not in PPI-specific cells. There was a modest negative correlation between CD25 expression level on Tregs and CD4 $TIGIT^+PD\text{-}1^+$ TConv cells in both total and viral-specific cells (r=-0.423, r=-0.394), but this relationship did not translate to PPI-specific cells. Since the IL2/IL-2R pathway modulates Treg, TfH, and CD25 expression, these findings suggest dysregulation in IL-2 signaling in PPI-specific T cells in T1D. We found comparable levels of IL-2 production in PPI-specific T cells from health controls and individuals with T1D but IL-2 production correlated with increased activation in PPI-specific cells from healthy controls but this was absent in in T1D. Together, our data suggests that autoreactive T cells in T1D are dysregulated in IL-2/IL-2R signaling as it relates to Tfh, Tregs, and activation, highlighting the importance of deeper functional probing beyond enumeration. The IL-2/IL-2R pathway is already being targeted in T1D trials and this work suggests it will be important to understand the effect on A-S cells to inform better therapies.

W127. MEK/ERK Mediated Diffuse Alveolar Hemorrhage in Murine Lupus

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Abstract Text: Introduction/Rationale About 3% of patients with lupus develop severe diffuse alveolar hemorrhage (DAH) with pulmonary vasculitis. C57BL/6 (B6) mice with pristane-induced lupus also develop DAH, but BALB/c mice are resistant. DAH is independent of Toll-like receptor signaling and other inflammatory pathways. This study examined the role of the MEK1/2 pathway Methods B6 and BALB/c mice were treated with pristane with or without inhibitors of MEK1/2 (trametinib/GSK1120212 [GSK]), ERK1/2 (SCH772984 [SCH]), JNK, or p38. Effects on lung hemorrhage and hemostasis were determined Results GSK and SCH abolished DAH, whereas JNK and p38 inhibitors were ineffective. Apoptotic cells were present in lung samples from pristane-treated mice but not in mice receiving pristane and GSK, and endothelial dysfunction was normalized. Expression of the ERK1/2- regulated transcription factor early growth response 1 increased in pristane-treated B6, but not BALB/c, mice and was normalized by GSK. Pristane also increased expression of the anticoagulant genes Tfpi and Thbd in B6 mice. The ratio of Tfpi to tissue factor (F3) to Tfpi increased in B6 (but not BALB/c) mice and was normalized by GSK. Circulating thrombomodulin protein levels increased in B6 mice and returned to normal after GSK treatment. Consistent with augmented endothelial anticoagulant activity, pristane treatment increased tail bleeding in B6 mice. Conclusion Pristane treatment promotes lung endothelial injury and DAH in B6 mice by activating the MEK1/2-ERK1/2 pathway and impairing hemostasis. The hereditary factors determining susceptibility to lung injury and bleeding in pristane-induced lupus are relevant to the pathophysiology of life-threatening DAH in systemic lupus erythematosus and may help to optimize therapy.

W128. VitD3tolDC Generates *in vitro* CD25$^+$ FOXP3$^+$ High T Regulatory Cells in MS Patients

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FUNDACIÓ INSTITUT D'INVESTIGACIÓ EN CIÈNCIES DE LA SALUT GERMANS TRIAS I PUJOL (IGTP), Barcelona, Catalonia, Spain

Abstract Text: Introduction T regulatory cells (Treg) play an important role in immune modulation and restoration of immune tolerance, emerging as a promising therapeutic strategy for the treatment of autoimmune diseases such as Multiple Sclerosis (MS). Our aim is to demonstrate tolerogenic dendritic cells differentiated in presence of VitD3 (VitD3tolDC) represent a promising tool for induction of antigen-specific Tregs *in vitro*. Methods VitD3tolDCs were generated by isolating CD14$^+$ monocytes from peripheral blood and inducing differentiation with GMCSF$^+$IL4 in the presence of VitD3 (tolerogenic agent). Phenotypic and proliferation assays were used to confirm the functionality of resulting VitD3tolDCs. To assess their impact on Treg induction, VitD3tolDCs were pulsed over-night with specific antigen, and co-cultured with autologous nave CD4$^+$ T cells for 8 days and IL-2 supplementation. Then, induced CD4$^+$ Tregs were collected to evaluate their phenotype and functionality. Results VitD3tolDCs exhibited a tolerogenic phenotype characterized by lower expression of CD83 and CD86, and reduced induction of autologous proliferation up to 50% compared to mature DCs (mDCs) differentiated in absence of VitD3. When analysing CD4$^+$ Naïve T cells, those cocultured with VitD3tolDC exhibited a higher % of CD25$^+$ FOXP3$^+$ high T regulatory cells in both healthy controls and MS patients. Moreover, when performing functional assays, Tregs from VitD3tolDC cocultures showed suppressive capacity. Conclusion VitD3-tolDCs effectively generate *in vitro* CD4$^+$ Tregs with suppressive capacity. These findings support the utility of VitD3tolDC as a platform for promoting Treg differentiation, highlighting its potential therapeutic relevance for MS.

W129. S-4321, a First-in-class Dual-cell Bifunctional PD-1:FcγRIIB Selective Agonist Antibody That Preserves PD-1 Expression, Prevents Fc-mediated Depletion of Target Cells, and Enhances Inhibitory Receptor Expression

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Abstract Text: Background: Inhibitory receptors (IR) upregulated on activated lymphocytes play a critical role in restoring and maintaining immune homeostasis to control autoimmunity. PD-1 is a well-characterized IR expressed on activated effector and regulatory T cells. Strong PD-1 agonism requires super-clustering, which is achieved by antibodies binding to Fc gamma receptors (FcγR) on APCs. Several PD-1 targeted antibodies have been investigated in autoimmunity with mixed clinical results. Rather than agonizing PD-1, these first-generation PD-1 antibodies primarily deplete PD-1$^+$ T cells, as demonstrated in human trials. S-4321 is a novel bifunctional antibody that agonizes the inhibitory PD-1 checkpoint receptor on T cells through a different binding site than its natural ligands, PD-L1 and PD-L2, and selectively engages and signals through the inhibitory FcγRIIb on B cells/APCs. Therefore, S-4321 is expected to restore immune homeostasis in cell-mediated autoimmunity. Methods: PD-1 expression was measured on activated T cells after culturing with S-4321 or benchmark PD-1 antibodies. FcγRIIb selectivity of the S-4321 Fc domain was demonstrated via binding assays. The lack of proinflammatory activity of the S-4321 Fc domain was assessed using TNFa production from activated monocyte-derived dendritic cells or antibody-dependent cellular cytotoxicity (ADCC) of human Tregs. S-4321 was tested in Treg differentiation assays, in naïve mice and disease models (GvHD). Results: S-4321 achieves prolonged agonism by binding to PD-1 with low affinity, which preserves PD-1 expression on T cells *in vivo*, unlike first generation PD-1 depleters. S-4321 also selectively binds and signals through FcγRIIb, avoiding the induction of proinflammatory cytokines and undesirable depletion of PD-1$^+$ T cells, such as Tregs, by ADCC through engagement of activating FcγR. S-4321 enhances induced Treg differentiation *in vitro*. *In vivo*, S-4321 reduces T cell expansion and proinflammatory cytokine

production in a murine model of GvHD and induces TIGIT expression on the surface of PD-1$^+$ T cells in naïve mice. Conclusion: In contrast to first generation PD-1 depleters, treatment with S-4321 does not result in loss of PD-1 expression on T cells, induction of proinflammatory cytokines, or depletion of PD-1$^+$ Tregs. By coupling PD-1 agonism with inhibitory FcγRIIb engagement, S-4321 has the potential to restore immune homeostasis in cell-mediated autoimmunity.

W130. Involvement of T-bet in the Pathogenesis of TLR7 Agonist–induced Lupus Model Mice

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Abstract Text: [Objective] To investigate the role of T-bet in systemic lupus erythematosus (SLE) using an imiquimod (IMQ)-induced murine model. [Methods] SLE-like pathology was induced by IMQ. The following analyses were performed. 1) Anti–dsDNA IgG, urinary protein, renal pathology, and glomerular immune complex deposition were compared between wild-type (WT) and T-bet knockout (KO) mice. 2) Splenic T- and B-cell subsets were analyzed by flow cytometry (FCM) in WT and KO mice. 3) Splenic CD4$^+$ T cells were stimulated *in vitro*, and IFNγ production was analyzed by FCM in WT and KO mice. 4) Anti–dsDNA IgG, urinary protein, renal pathology, and glomerular immune complex deposition were compared among CD4 T-bet conditional knockout (CD4 cKO), CD19 T-bet conditional knockout (CD19 cKO) , and T-bet floxed (Flox) control mice. 5) Splenic T- and B-cell subsets were analyzed by FCM in Flox, CD4 cKO, and CD19 cKO mice. 6) Splenic CD4$^+$ T cells were stimulated *in vitro*, and IFNγ production was analyzed by FCM in Flox, CD4 cKO, and CD19 cKO mice. [Results] 1) Anti–dsDNA IgG titers and glomerular immune complex deposition were reduced in KO mice compared with WT mice, and urinary protein levels showed a decreasing trend; however, renal histopathology did not differ. 2) T peripheral helper cells, T follicular helper cells, and age-associated B cell (ABC)-like cells were reduced in KO mice. 3) CD4$^+$ T cells from KO mice exhibited reduced IFNγ production. 4) Anti–dsDNA IgG levels tended to decrease in CD4 cKO mice, whereas no changes were observed in CD19 cKO mice. No significant differences were detected in urinary protein, renal histopathology, or glomerular immune complex deposition in either group. 5) ABC-like cells were reduced in CD4 cKO mice but not in CD19 cKO mice, while T Cell subsets were comparable among Flox, CD4 cKO, and CD19 cKO mice. 6) CD4$^+$ T cells from CD4 cKO mice showed reduced IFNγ production, whereas no significant changes were observed in Flox or CD19 cKO mice. [Conclusion] T-bet may contribute to SLE pathogenesis by regulating IFNγ production in CD4$^+$ T cells and promoting B-cell differentiation into ABCs.

W131. Regulatory Role of IL-32 and Its Feedback Loop in Cytokine Network- a New Dimension in Autoimmune Rheumatologic Diseases

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Abstract Text: IL-32 Is a proinflammatory cytokine; in human IL-32 can establish a positive feedback loop with other proinflammatory cytokines such as TNF, IL6,IL-8, IL-17. Limited studies are available on IL-32 in human diseases. Here we have explored the cellular/molecular mechanisms of IL-32 in respect to synovial inflammation and its interactions with critical cytokines of autoimmune diseases (AD) such as TNF,IL-6,IL-8,IL-17.Here we determined the functional role IL-32 on Synovial fibroblasts(FLS) proliferation and on induction of inflammatory cytokines/endopeptidase (IL-6,IL-8,MMP3) related to synovial inflammation and expression of RANK-L/osteoclast activation. FLS isolated from psoriatic arthritis(PsA)/rheumatoid arthritis(RA) (n=5 each) were cultured with IL-32γ and IL-32α (100 ng/ml) for 5 days. For proliferation assay MTT/CFSE dilution assays were done. To determine the effect of IL-32γ/IL-32α on the production of relevant cytokines/chemokines/endopeptidase the FLS culture supernatants were evaluated for IL-6,IL-8,MMP3 and RANK-L by specific ELISA kits. Similar experiments were done

with IL-17A (100 ng/ml),TNF-α (20ng/mL). Concentrations of IL-32γ/IL-32α, IL-17 A, TNF-α were selected by doing dose response analysis. Level of IL-32 was measured in synovial fluid (SF) of PsA, RA and osteoarthritis(OA) by ELISA. Compared to IL-32α, significant effect on FLS biology was observed with IL-32γ. Among the subtypes of IL-32 (α,β,γ) IL-32γ is the most potent inflammogenic. In MTT assay we observed rIL-32γ induced significant proliferation in PsA/RA FLS (OD: 0.38±0.08/0.34±0.05)) compared to FLS only cultured with media (OD: 0.25±0.04; p< 0.001). Compared to rIL-32γ alone stimulation with rIL-32γ+rIL-17A induced significantly more proliferation of FLS (p< 0.001). Furthermore, FLS treated with rIL-32γ +TNF-α+rIL-17A had highest proliferation. These results suggest the effect of rIL-32γ in the cytokine network of synovial inflammation in AD. Also, PsA/RA FLS stimulated with rIL-32γ produced significantly more IL-6, IL-8 MMP3 and RANK-L compared to media (p< 0.001). The amount of IL-32 in the SF of PsA (980±30 pg/ml), RA ((430±50 pg/ml) was significantly higher compared to OA (70±30 pg/ml)(p< 0.001). Results of this study substantiates contributing role of IL-32 in synovial inflammation, osteoclast activation and provide new insights in the pathogenesis of AD such as in RA, PsA; and IL-32 is likely to be novel target for treatment of these conditions.

W190. Unraveling Childhood-onset Systemic Lupus Erythematosus (cSLE) Using High-dimensional Data: An Immunology-based Diagnostic Model to Enhance Theragnosis and Mechanistic Insights

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Abstract Text: Childhood-onset systemic lupus erythematosus(cSLE) presents diagnostic challenges due to its heterogeneous nature. We aim to resolve this immunopathogenic complexity with high-dimensional mass cytometry (CyTOF) to study the lupus immunome. Using machine learning, we developed a diagnostic model utilizing mechanistically relevant cell clusters distinguishing patients from controls, validated with an independent cSLE cohort. The discovery cohort examined peripheral blood mononuclear cells (PBMCs) from 26 cSLE patients (53 timepoints, median age: 14 years) and 17 age-matched healthy controls using a 43-marker CyTOF panel. Quality check, cell clustering and phenotypic annotation were done using our unsupervised Extended Poly-dimensional Immunome Characterisation(EPIC) pipeline. Machine learning algorithms [linear SVM, PLS-DA, random forest (RF) using MetaboAnalyst 6.0] identified predictive cell clusters for cSLE to ensure model robustness. Cell frequencies are shown as percentages of total CD45+ PBMCs with median and interquartile range (IQR), with statistical significance set at p< 0.05(Mann-Whitney U). Validation was conducted on a new cohort of 18 cSLE patients (median age: 11.5) and 23 age-matched controls. 67 unique cell clusters (26 were significantly different) were used to map the validation cohort. Mapping was automated based on previous expert annotation of the derived cell clusters from unsupervised analysis of discovery data. Validation cohort clusters mirrored accurately the phenotypes of the discovery cohort cell populations. Interestingly, there were increased memory Tregs in cSLE (cSLE vs. healthy: 1.16[0.79-1.92]% vs. 0.48[0.32-0.80] %, p< 0.0001) but no changes in naive Tregs. Memory Treg-like populations (i.e. CD3+CD4+CD45RO+CD25-Foxp3+CTLA+) were also higher in cSLE than healthy (2.75[1.90-4.36] vs. 1.28[0.83-1.78], p< 0.0001). Concurrently, CD8+CD45RA+BAFF+ T cells were raised in cSLE (8.91[6.78-11.4] vs. 3.22[2.79-4.86], p< 0.0001). Ten immunological features were selected through ranking of the most important features generated by machine learning algorithms on discovery cohort data. Average accuracy of discriminating cSLE and healthy based on 100 cross validations (with linear SVM, PLS-DA and RF algorithms) in the discovery cohort is 85.4%(median), (IQR 83.3-85.6%). Sensitivity for the validation cohort is 88.9% (83.3-100%). Further studies will quantitate autoantibody titers (anti-c1q/-nucleosome/-dsDNA/-Smith) for comparison. The ten selected features reflect broad changes in the lupus immunome: perturbed immunoregulation, cytokine production and reduced immune activation threshold. This aids our understanding of SLE immunopathogenesis and can reinforce current diagnostic criteria

Autoinflammatory Diseases

Th138. Bacterial Triggers of Autoimmunity: Borrelia Peptidoglycan in Post-Treatment Lyme Disease

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Abstract Text: Persistent symptoms after infection remain a major clinical challenge, yet the immune mechanisms driving these syndromes are not well defined. Post-Treatment Lyme Disease (PTLD) affects over one million individuals in the United States and is characterized by chronic fatigue, musculoskeletal pain, and cognitive dysfunction despite appropriate antibiotic therapy. The immunopathogenesis underlying PTLD is poorly understood. We analyzed circulating immune cells from PTLD patients and identified a hyperinflammatory monocyte subset enriched in the PTLD cohort. These monocytes exhibited transcriptional programs associated with autoimmunity, including heightened TNF signatures, antigen-presentation pathways, and inflammatory cytokine production. Additional immune abnormalities—such as increased Th17 cells, expanded $CD8^+KIR^+$ regulatory T cells, and dysfunctional $CD4^+FOXP3^+$ Treg signatures—mirrored features observed in established autoimmune diseases. Because Borrelia burgdorferi peptidoglycan (PGBb) is known to persist in the host after bacterial clearance, we tested whether PGBb could induce PTLD-like immune states. Stimulation of healthy human monocytes with purified PGBb recapitulated the PTLD monocyte transcriptomic phenotypes. Using human spleen immune organoids, we further demonstrated that PGBb activates autoreactive B cells and triggers proinflammatory programs across multiple immune populations. Strikingly, peptidoglycans from bacterial species implicated in rheumatoid arthritis flares induced comparable inflammatory responses. These findings reveal a potential unifying mechanism for PTLD: persistence of bacterial peptidoglycan can rewire innate and adaptive immune responses, driving chronic inflammation and promoting autoreactivity even after infection is cleared. More broadly, our work suggests that species-specific peptidoglycans may act as previously under-appreciated microbial triggers of autoimmune disease and post-infectious syndromes.

Th139. Neutrophil-targeted Colchicine Delivery via CD177-conjugated Nanolipogels Ameliorates Disease in Familial Mediterranean Fever

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Abstract Text: Background: Familial Mediterranean fever (FMF) is an autoinflammatory disease caused by dysregulated innate immunity, in which neutrophils play a central pathogenic role. Although colchicine is the first-line therapy for FMF, its efficacy is sometimes limited, and systemic administration may cause dose-dependent gastrointestinal toxicity. A neutrophil-targeted drug delivery system (DDS) may enhance therapeutic efficacy by selectively delivering anti-inflammatory agents to disease-relevant cells. CD177 is a neutrophil-specific surface marker and an attractive target for neutrophil-directed DDS. In this study, we evaluated the therapeutic potential of anti-CD177 antibody–conjugated nanolipogels (NLG) in an FMF mouse model. Methods: Rhodamine-labeled nanolipogels were generated and conjugated with either an isotype control antibody or an anti-CD177 antibody. Selective uptake of rhodamine-labeled NLG by neutrophils was identified by flow cytometry. Therapeutic efficacy was assessed using Human MEFV encoding M694I knock-in (Mefv^M694I/M694I) mice. Experiments were initiated at 8 weeks of age, with intravenous treatments administered three times weekly for 4 weeks. Mice were divided into

four treatment groups: phosphate-buffered saline (PBS) group (n = 10), free colchicine group (n = 10), isotype control antibody–conjugated colchicine-containing NLG group (n = 11), and anti-CD177 antibody–conjugated colchicine-containing NLG group (n = 10). Free colchicine was administered at 0.05 mg/kg, and the colchicine content encapsulated in NLG was adjusted to the same equivalent dose. Body weight was monitored longitudinally as an objective indicator of systemic inflammation and disease severity. Results: Flow cytometric analysis demonstrated selective uptake of rhodamine-labeled nanolipogels by neutrophils, confirming antibody-mediated neutrophil targeting. In the FMF model, mice in the PBS group showed attenuated age-associated body weight gain, consistent with previous reports. Neither free colchicine nor isotype control antibody–conjugated NLG improved body weight trajectories compared with PBS. In contrast, improvement in body weight gain was observed exclusively in the anti-CD177 antibody–conjugated NLG group, indicating that CD177-mediated neutrophil targeting was required to achieve a measurable *in vivo* therapeutic effect. Conclusions: Anti-CD177 antibody–conjugated nanolipogels, validated for neutrophil-selective uptake by flow cytometry, improved inflammation-associated impairment of body weight gain in Mefv^M694I/M694I mice, whereas free colchicine and non-targeted NLG did not. These findings support CD177-mediated neutrophil targeting using NLG as a promising therapeutic strategy for FMF.

Th141. Longitudinal Single-cell Transcriptomic Profiling Reveals Persistent Inflammatory Phenotype in Intravitreal rAAV-induced GTAU

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Abstract Text: Objective Recombinant adeno-associated virus (rAAV) is the most clinically advanced platform for ocular gene therapy. However, anti-vector immune responses may cause gene therapy-associated uveitis (GTAU), which is a critical barrier to therapeutic efficacy. Conventional flow cytometry provides limited resolution of cellular phenotypes and communication networks that arise during GTAU. We hypothesized that longitudinal single-cell RNA-Seq would allow comprehensive mapping of immune populations and their functional states after intravitreal (IVT) rAAV treatment. Methods GTAU was induced in wild-type C57BL/6 mice by IVT injection of rAAV2/7m8.CMV.eGFP:WPRE at 3.3E10 gc/eye. Single eyes were harvested at 14, 29, and 58 days post-injection (dpi) and processed via enzymatic digestion. $CD45^+$ cells were purified by FACS for sequencing via the GEM-X Flex Gene Expression platform (10x Genomics). A control dataset (Cui et al., 2023; GSE202186) was integrated, and quality control, analysis of clustering, differential gene expression, and cell-to-cell communication were performed using CellRanger, Seurat, and CellChat. Results Single-cell profiling at 14 (n = 41,995), 29 (n = 12,511), and 58 (n = 18,181) dpi revealed diverse immune cell infiltration in rAAV-treated eyes. We identified significant populations of T cells ($Cd4^+$, $Cd8a^+$, Tcrg-$V6^+$, and $Mki67^+$), NK and NK T cells, B cells, cDC/pDCs, monocytes/macrophages, and microglia. While infiltration of NK cells (35.15%) was similar to T cells (37.06%) at 14 dpi, the NK cell population relative to total cell number diminished over time, in contrast to the T cell population (27.79% vs. 47.7% at 58 dpi). A $CD8^+$ effector memory population was present at 14 dpi, (18%) accompanied by a Th1-dominant $CD4^+$ T cell response (46%). We noted substantial increase in $CD4^+$ T cell exhaustion phenotype over time (2% at 14 dpi; 27% at 58 dpi), but no increase in Treg cells. Notably, ICAM/JAM/Galectin adhesion molecules and CCL/CXCL chemokine networks remained persistently active, suggesting maintained recruitment and retention of immune cells. Conclusions We longitudinally characterized IVT GTAU with ssRNA seq analysis. rAAV-treated eyes demonstrated persistent T cell populations through 58 dpi. While NK cells likely contribute to the early response, their relative decline suggests that sustained inflammation is driven by the remaining T cell population.

Th142. Functional Validation of Variants of Unknown Significance in COPA Syndrome

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Abstract Text: BACKGROUND: COPA syndrome is an autosomal dominant autoinflammatory disorder causing childhood-onset of interstitial lung disease, autoimmunity, arthritis, and renal disease. Disease-causing mutations cluster in the WD40 domain of coatomer subunit α (COPA), damaging its ability to recognize proteins with C-terminal dilysine motifs for retrograde trafficking from the Golgi to Endoplasmic Reticulum. Disease is specifically caused by Golgi accumulation and activation of the innate immune sensor STING (stimulator of interferon genes), with patients demonstrating activation of type I interferons, secretion of nuclear factor kappa B (NFkB) dependent cytokines, and increased ER stress. Clinical sequencing has identified COPA variants of unknown significance (VUS) in individuals with a wide range of phenotypes, including mutations unlikely to impair COPA function or cause COPA syndrome. We optimized quantifiable assays to functionally evaluate COPA VUS. METHODS: Plasmids encoding COPA VUS were co-transfected with STING into human STING-deficient HEK293T cells. COPA-dependent activation of STING-dependent pathways was determined for each VUS using luciferase assays measuring induction of interferon β (IFNβ), NFkB, Activating Transcription Factor 6 (ATF6) as a readout of ER stress, and ATF4 as a readout of the related integrated stress pathway. The ability of COPA VUS to recognize known COPA-binding dilysine peptides was quantified using flow cytometry of peptide coated beads. RESULTS: Transfection assays were optimized using WT and E241K COPA, a well-validated pathogenic variant. Known pathogenic variants of COPA were tested to determine pathogenic ranges of COPA-dependent activation of IFNβ, NFkB, ATF6, and ATF4. COPA VUS results were compared to these ranges, allowing classification as pathogenic (impaired dilysine binding + increases of all 3 reporters), likely pathogenic (impaired binding + increase of 2 reporters), non-COPA syndrome disease (intact binding + increase of 1 or more reporters), and likely benign (intact binding without increases in reporters). CONCLUSIONS: We created and validated functional assays for COPA VUS with which we are testing over 40 variants found in individuals whose phenotypes demonstrate variable overlap with COPA syndrome. Some variants tested, including three WD-40 domain variants, are well tolerated. VUS classification will inform patient care by clarifying whether identified COPA mutations are expected to cause disease.

Tu137. STAP-1-derived Peptide Suppresses TCR-mediated T Cell Activation and Ameliorates Autoimmune Diseases by Inhibiting STAP-1/LCK Binding

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Abstract Text: Signal-transducing adaptor protein-1 (STAP-1) is an adaptor protein highly expressed in secondary lymphoid tissues such as the spleen and lymph nodes. It contains a pleckstrin homology (PH) domain in its N-terminal region and a Src homology 2 (SH2) domain in its C-terminal region. We previously reported that STAP-1 functions as a scaffold protein to promote T cell receptor (TCR)-mediated signaling by interacting with LCK, ITK, and phospholipase C (PLC)-γ1. Based on these findings, we hypothesized that synthetic peptide which inhibits STAP-1/LCK binding could suppress TCR-mediated T cell activation and applies to a treatment of T cell-mediated autoimmune diseases. In this study, we synthesized a series of peptide targeting LCK-binding sequence of STAP-1 and did phenotypically screening that suppresses T cell proliferation. Next, we identified a hit peptide and optimized it (which we name as “iSP1”). After that, we evaluated its effects on human and murine T cells. iSP1 effectively inhibited STAP-1/LCK binding and suppressed TCR-mediated signal transduction, interleukin-2 production, and T cell proliferation. Also, we examined whether it has a therapeutic efficacy in a mouse model of T cell-mediated autoimmune diseases. We employed experimental autoimmune encephalomyelitis (EAE) mice as a model of Multiple Sclerosis. iSP1-administeredtreated mice exhibited significantly mitigated disease development by reduced infiltration of Th1 and Th17 cells into the central nervous system. These findings suggest that iSP1 has potential as a novel immunomodulatory agent for the treatment of T cell-mediated autoimmune diseases.

Tu138. Proteomic Profiling of Interstitial Fluid from CindU and CSU Patients Highlights the Presence of Shared MRGPRX2 Ligands and Inflammatory Biomarkers

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Abstract Text: Mast cells are central effectors of many chronic inflammatory skin diseases. MRGPRX2, a receptor expressed on mast cells and sensory neurons, activates mast cells in response to cationic ligands and has been implicated in the pathogenesis chronic inducible urticaria (CIndU) and chronic spontaneous urticaria (CSU). However, disease biology remains incompletely understood due to limited access to lesional skin tissue and skin-relevant biofluids. Additionally, peripheral blood analyses often fail to reflect localized cutaneous inflammation. To directly interrogate the lesional microenvironment, we collected dermal interstitial fluid (dISF) from the skin using a novel microneedle device. In cold CIndU patients, dISF was obtained from non-lesional skin and from lesional skin at 15 minutes and 4 hours following cold provocation. In CSU patients, dISF was collected from both lesional and non-lesional sites. Proteomic profiling was performed using mass spectrometry and Olink analyses. Lesional dISF from both CIndU and CSU patients showed increased levels of mast cell activation markers, including tryptase and CD107a, compared to non-lesional skin. Importantly, endogenous MRGPRX2 ligands such as LL-37, PAMP, and Cathepsin S were enriched in lesional samples. Olink analysis revealed elevated cytokines and chemokines associated with immune cell recruitment and amplification of inflammation in lesional skin. Notably, these proteomic signatures were consistently observed across both CIndU and CSU. Together, our findings support a model in which MRGPRX2-driven mast cell activation represents a shared pathogenic mechanism underlying both diseases.

Tu139. Targeting MRGPRX2 Addresses Unmet Need in Atopic Dermatitis: An in Silico Network Analysis

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Abstract Text: While IL-13/IL-4 inhibitors have transformed atopic dermatitis (AD) treatment, 30–40% of patients remain partial or non-responders. We investigated the therapeutic potential of targeting MRGPRX2, a mast cell and sensory neuron specific receptor, using a predictive computational engine to define its role in AD pathogenesis and its overlap with Type 2 immunity. We developed a MRGPRX2-specific gene signature from RNAseq data of human mast cells activated with multiple ligands in the absence or presence of the oral MRGPRX2 inhibitor EVO756. This signature was integrated into the CytoReason AD in silico disease model—a comprehensive computational representation of disease biology that integrates 24 RNAseq datasets, >2,000 human skin samples including clinical EASI scores and dupilumab response data. The MRGPRX2 signature was highly enriched in lesional AD tissue and associated with EASI scores. Disease features association analysis identified MRGPRX2 as a central "hub" linking T Cell effector function (Th2, Th17, Th22), neuroinflammation, pruritus, and barrier dysfunction. While MRGPRX2 and IL-13/IL-4 signatures share biology in the Th2 and neuroinflammatory space, MRGPRX2 uniquely covers "white space" pathways not addressed by IL-13/IL-4 inhibition. Critically, while dupilumab significantly reduced its primary target signatures, the MRGPRX2 signature remained largely unimpacted in responders and was found to be active in non-responders. These data position MRGPRX2 as a critical pathway in AD, which persists despite standard-of-care biologics treatment. Targeting MRGPRX2 via oral inhibitors like EVO756 offers a compelling strategy to address the significant unmet need in both Type 2-high and Type 2-low AD populations.

W132. Leveraging a Proteomics Data Lake for Immune-mediated Disease Insights

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Abstract Text: With the availability of state-of-the-art high-throughput protein detection capabilities, Proteomics has become an essential tool in understanding disease mechanisms, identifying novel therapeutic targets and biomarkers, and indication selection and expansion for drug(s) positioning. At Sanofi, we have developed a Proteomic Data Lake (PDL) using high-quality proteomic datasets sourced from internal experiments and published studies, providing novel insights across immune-mediated conditions with the potential to accelerate multiple phases throughout the drug discovery process. A computational analytical platform to evaluate proteome interactions within and across immune diseases from multiple independent cohorts could provide deep, unbiased insights on a human-first, real-world basis to expedite drug development for target discovery and biomarker identification. While PDL has indeed proven valuable to credential protein-indication pairs, to identify shared pathophysiological mechanisms across immune-mediated diseases, we employed in silico connectivity score (CS) to systematically compare between disease signatures spanning diseases across different tissues/organs. Using weighted connectivity scores based on Kolmogorov-Smirnov enrichment statistics such as CS, we systematically analyzed disease similarities across >11M differential protein abundance tests. Our analysis confirmed established clinical relationships while revealing novel associations: obesity showed expected strong connectivity with type 2 diabetes (CS=1.29), sleep apnea (CS=1.37), and cardiovascular diseases including atherosclerosis (CS=1.24) and heart failure (CS=1.28). Additionally, we uncovered significant connectivity of obesity with autoimmune conditions, supporting emerging understanding of metabolic-immune crosstalk. This comprehensive immuno-proteomic resource demonstrates the power of systematic disease signature comparisons for advancing precision medicine approaches, revealing shared molecular mechanisms and potential therapeutic repositioning opportunities across diverse inflammatory and autoimmune conditions for protein-disease associations to predict novel therapeutic targets, identify patient stratification biomarkers, and optimize indication selection across the immune disease landscape.

W133. Defining a Signature of Autoreactive CD8⁺ T Cells in HLA-B27-associated Autoimmune Disease

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Abstract Text: The Human Leukocyte Antigen B27 (HLA-B27) allele is associated with multiple autoimmune diseases, including Ankylosing Spondylitis (AS), Acute Anterior Uveitis (AAU), Psoriatic Arthritis (PsA), Reactive Arthritis, and Enteropathic Arthritis (EnA). Recent work in AS and AAU has identified pathogenic CD8⁺ T cells with a shared T cell receptor (TCR) motif that cross-react to intestinal bacterial antigens and autoantigens presented by HLA-B27. We investigated whether additional properties of pathogenic CD8 T cells are also shared across HLA-B27-associated diseases. We analyzed public single-cell RNA and TCR sequencing data of CD8⁺ T cells, spanning multiple HLA-B27⁺ diseases (AS, AAU, and PsA), tissues (blood, joint, and eye), and parent institutions (Washington University in St. Louis, King's College London, University of Toronto, University Hospital LMU Munich). In parallel, we performed single-cell TCR and gene expression sequencing on HLA-B27⁺ healthy controls and HLA-B27⁺ patients with AAU, AS, and/or IBD. We detected pathogenic CD8⁺ T cells in the blood, joint (AS & PsA), and eye (AAU), suggesting a generalizable role across HLA-B27-associated autoimmunity. Pathogenic CD8⁺ T cells were also present in the blood and gut of patients with HLA-B27⁺ IBD, consistent with an enteric origin. Our gene expression analysis also revealed that the pathogenic cells were transcriptionally distinct from conventional effector CD8⁺ T cells, expressing markers of Type 17 immunity (KLRB1, CEBPD) and tissue homing (CCR6) and forming their own cluster within the single-cell data. The pathogenic cells also displayed a shared biology across contexts,

clustering together regardless of which disease, tissue, or institution the data came from. To further demonstrate the pathogenic cells' distinct gene expression profile, we curated a pathogenic gene signature from our in-house AS/AAU dataset and then applied the signature to our IBD data and the public datasets. In all cases, the gene signature strongly associated with the presence of canonical pathogenic TCR features, such as usage of the TRBV9 or TRBV5-5 gene. From our analysis, we conclude that the shared TCR motif of the pathogenic cells drives a pathogenic gene expression program which constitutes a shared underlying biology in HLA-B27-associated autoimmunity.

W134. Therapeutic Efficacy of a Novel Oral NRF2 Activator in a Murine Ulcerative Colitis Model

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Abstract Text: Introduction Ulcerative colitis (UC) arises from three interconnected pathological processes: impaired epithelial barrier function, persistent mucosal inflammation, and elevated oxidative stress. NRF2 (NF-E2–related factor 2), a transcription factor that serves as a central coordinator of cellular defense programs, orchestrates antioxidant defenses, inhibits inflammatory pathways, and facilitates epithelial regeneration. Beyond its cytoprotective functions, NRF2 activation regulates intestinal immunity and influences UC development. To address the multifactorial drivers of UC, we have developed a novel, potent, and selective oral NRF2 agonist that shows efficacy in a preclinical model of UC. Methods MTAI-1025 was evaluated for *in vivo* efficacy in a 14-day DSS-induced mouse colitis model. Mice received compound (30, 60, or 100 mg/kg, PO, daily from days -1 to 12), vehicle, or a reference anti-IL-12p40 monoclonal antibody (10 mg/kg, IP on days 6 and 9). Inhibition of immune-related signaling was determined in LPS/IFN-g-stimulated monocyte-derived DCs. MoA studies were conducted using HEK293 cells stably transfected with FLAG-NRF2 and HA-KEAP1 (wt or C151) or HEK293 cells stably transfected with split luciferase tagged Cul3 or NRF2 and KEAP1. Results In the DSS model, MTAI-1025 dose-dependently reduced DAI and body weight loss. Treatment also improved mucosal architecture, decreased inflammatory cell infiltration, and lowered pro-inflammatory cytokine levels (IL-1β, IL-6, CXCL1) compared with controls. Dose-dependent activation of the NRF2 transcriptional pathway was confirmed by induction of NQO1 mRNA. MTAI-1025 reduced cytokine secretion in MoDC cells and specifically regulated the dynamics of the NRF2/KEAP1/Cul3 complex at KEAP1 C151. Conclusion These results demonstrate that MTAI-1025 effectively attenuates inflammation and exhibits efficacy in an *in vivo* model of UC, supporting its potential as a first-in-class oral NRF2-targeted therapy for UC.

W135. Validating the "Bat Engine" for Target Discovery: Discovery of PS-1001, a First-in-Class Pan-inflammasome Inhibitor Inspired by Evolutionary Biology

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Abstract Text: Bats have evolved superior control of inflammation to survive the metabolic stress of flight and high viral loads without immunopathology. The Paratus "Bat Engine" platform systematically interrogates this unique biology to identify novel targets for immunology and inflammation (I&I). This translational potential is exemplified by our lead asset, PS-1001. Our analysis identifies the adaptor protein ASC as a central regulatory hub, evidenced by mutations in bat ASC2 and extensive evolutionary adaptations throughout the IL-1 superfamily in bats. We hypothesized that therapeutically targeting this node could unlock bat-like inflammation control in humans. To translate this insight, we engineered PS-1001, a VHH-Fc fusion protein designed for cellular internalization.

Mechanistically, PS-1001 exerts a dual effect: inhibiting the nucleation of productive ASC specks and actively disassembling pre-formed filaments, thereby abrogating signal propagation. PS-1001 binds ASC with high affinity, demonstrating cellular internalization and target engagement in activated PBMCs. *In vivo*, it exhibited robust efficacy, ameliorating pathology in murine models of gout, psoriasis, and colitis. In Hidradenitis Suppurativa (HS) patient skin explants (HS 3D-SeboSkin model), PS-1001 reduced IL-18 secretion by >60% and suppressed a broader inflammatory signature than adalimumab. The molecule displays favorable physicochemical properties for subcutaneous injections, with PK modeling supporting >Q2W dosing. Completed non-GLP toxicology studies in mice and non-human primates support its translational safety profile. PS-1001 validates ASC as a therapeutic target. We are advancing HS as our lead indication toward First-in-Human trials in late 2026, while exploring the broad clinical potential of PS-1001 in other inflammatory diseases.

W136. Single-cell RNA Sequencing of Human Bone Marrow Reveals Pathological Immune Landscapes and Unique Erythroid/B-cell Populations in Macrophage Activation Syndrome

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Abstract Text: Background: Macrophage activation syndrome (MAS) is a life-threatening inflammatory condition complicating Still's disease (SD). Despite its severity, systematic characterization of the human bone marrow (BM) immune landscape at the single-cell level remains limited, preventing a comprehensive understanding of its pathogenesis. Objective: To examine disease-associated immune cell populations and inflammatory signaling features in the BM of MAS patients with SD using single-cell RNA sequencing (scRNA-seq). Methods: We performed scRNA-seq on BM mononuclear cells from 5 MAS patients with SD and 3 healthy controls. Data analyses included unsupervised clustering, pseudo-bulk differentially expressed gene (DEG) analysis, and Gene Set Enrichment Analysis (GSEA). Transcription factor (TF) activity was estimated using VIPER/DoRothEA, and intercellular communication was mapped via CellChat. Results: In addition to the expansion of inflammatory macrophages and $CD8^+$ T cell populations, we identified unique MAS-specific clusters within the erythroid and B-cell lineages. These populations showed altered expression of genes related to interferon (IFN) responses, cellular stress, and antigen presentation, accompanied by the activation of specific inflammatory signaling pathways. CellChat analysis indicated marked changes in BM cell–cell communication patterns, with inflammatory macrophages acting as prominent sources of signaling. Conclusion: MAS is associated with immune alterations affecting multiple hematopoietic lineages in the BM, extending beyond macrophage-T cell interactions to include erythroid and B-cell compartments. These findings expand current understanding of BM immune involvement in MAS complicating SD.

Basic Science of Immunology - Adaptive Immunity

Th143. Granzyme K+ CD8 T Cells Colocalize with Myeloid Cells and Activate Them Through Several Pathways

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Abstract Text: Objective: Granzyme K (GZMK)-expressing CD8 T cells are abundant in tissues from many different diseases, including malignancies and autoimmune conditions, but their roles in disease pathogenesis are unclear. GZMK+ CD8 T cells have many potential effector functions, including release of cytokines, chemokines, and GZMK, which can initiate complement cascades, generating anaphylatoxins C3a and C5a. In this project, we localize GZMK+ CD8 T cells within RA synovial tissue and characterize their molecular interactions with neighboring cells. Methods: Spatial transcriptomic data were collected from three samples of human synovial tissue from patients with RA using a custom 377-probe panel on the Xenium platform (10x Genomics). For *in vitro* experiments, CD14+ myeloid cells isolated from human peripheral blood mononuclear cells by magnetic-activated cell sorting and stimulated with recombinant or serum-purified GZMK, IFN, C3a, and/or C5a, for 24 hours and then analyzed by ELISA or RT-PCR. Results: In spatial transcriptomic data, GZMK+ T cells were preferentially located among myeloid cells in perivascular areas of the sublining in human RA synovial tissue. To investigate molecular interactions between GZMK+ CD8 T cells and myeloid cells, we stimulated CD14+ blood monocytes *in vitro* with soluble factors produced by GZMK+ CD8 T cells directly (GZMK, IFN) or via activation of complement cascades (C3a, C5a). We found that recombinant GZMK alone induced monocytes to produce CCL2, CCL3, and IL-6. GZMK did not induce CXCL10 or IL-1beta on its own, but it synergizes with IFN to induce robust release of these pro-inflammatory factors. GZMK and C5a had additive effects on IL-6 release. Conclusions: GZMK+ CD8 T cells localize near myeloid cells in the sublining of inflamed RA synovium. GZMK can directly induce monocytes to secrete CCL2, CCL3, and IL-6, and it can act together with IFN or through complement cascades to induce IL-1, IL-6, and CXCL10. These chemokines and cytokines form a positive feedback loop, recruiting and activating additional CD8 T cells and blood monocytes in inflamed RA synovium.

Th144. Analysis of the Expression of CD84 Isoforms in AML, CLL and T-ALL and Functional Implications

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Abstract Text: CD84 (SLAMF5) is a homophilic Ig-like receptor activated through interaction of its distal Ig-like domains. Its intracellular domain mediates signal transduction, and up to six isoforms have been described, differing in their intracellular structures. Most functional studies have focused on the canonical isoform c, whereas the expression and signaling properties of non-c isoforms remain poorly characterized. We analyzed CD84 isoforms at the RNA level in primary samples and/or cell lines from acute myeloid leukemia (AML), T Cell acute lymphoblastic leukemia (T-ALL), chronic lymphocytic leukemia (CLL) and Burkitt lymphoma, and compared them with their corresponding healthy hematopoietic counterparts. CD84 isoforms were detected, including isoforms lacking ITSM motifs (d, e) or the distal extracellular Ig-like V domain (ΔV). Isoform e was upregulated in AML, isoform c in T-ALL,

and isoforms c and e in CLL. To assess isoform functionality, isoforms c, d, and e were individually expressed in CD84-KO MOLM-13 (AML) and CD84-negative HEK293 cells. Phos-Tag analyses revealed λ-phosphatase-sensitive mobility shifts for all three isoforms, indicating phosphorylation and signaling competence. In silico kinase predictions suggested that isoform d may function downstream of Ca^{2+}-dependent pathways, whereas isoform e may be regulated by Src-family or STE kinases. ΔV isoforms reached the cell membrane but were predominantly retained intracellularly. Isoform c was preferentially incorporated into exosomes. Together, these findings provide the first comprehensive characterization of CD84 isoforms in hematologic malignancies, reveal previously unrecognized signaling capacities of non-c isoforms, and support the translational development of CD84-targeted therapies, including the forthcoming first-in-human clinical trial GYA-CART84 (EU trial No. 2024-519966-31-00).

Tu140. Endogenous Activation of $CD8^+$ T Cells Is Jointly Constrained by a MYB-dependent Program and Fas/FasL-mediated Deletion

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Abstract Text: Immune homeostasis is sustained by mechanisms that orchestrate the generation, differentiation, and death of lymphocytes. Just as immune cells are armed with effector functions to confront pathogenic threats, their timely and purposed disposal curbs collateral injury. The Fas/Fas ligand (FasL) system moderates immune responses by inducing apoptosis of activated lymphocytes. Genetic deficiencies of Fas or FasL cause a syndrome of chronic lymphoproliferation and autoimmunity in humans and mice. This disorder entails progressive accumulation of an unusual CD4− CD8− T cell population, which was recently dubbed Fas-Controlled (FC) T cells. Studies of these aberrant cells have presented conflicting models of their ontogeny and function which remain unresolved. Here we show that FC T cells originate from endogenous, T cell receptor (TCR)-driven activation of peripheral $CD8^+$ precursors. When Fas-deficient naïve $CD8^+$ T cells were transferred into unperturbed recipients, a fraction proliferated extensively to give rise to FC T cells. TCR sequencing of accumulating FC T cells in mice treated with FasL blockade revealed a broad repertoire comprising oligoclonal expansions. To test whether the TCR determines FC fate, we optimized a TCR-swap system in murine $CD8^+$ T cells that concurrently disrupts endogenous TCR chains via CRISPR/Cas9 and integrates a transgenic TCR via retroviral transduction. Expression of TCRs sequenced from FC but not naïve $CD8^+$ T cells was sufficient to direct FC differentiation *in vivo*. Transcriptional and chromatin accessibility profiling of FC T cells identified features consistent with anergy, including transcription factor activity that adapts $CD8^+$ T cells to chronic stimulation (Myb, Bach2, Id3) and lack of cytotoxic effector programs (Tbx21, Id2). Conditional deletion of Myb in $CD8^+$ T cells prevented their differentiation into FC T cells, while causing the emergence of a distinctive effector population following FasL blockade. Experiments are ongoing to examine whether $CD8^+$ precursors bound for FC differentiation instead become diverted toward a terminal effector fate without MYB. Finally, we found circulating FC T cell frequency to be transiently elevated in cancer patients receiving checkpoint blockade immunotherapy. Our study defines a regulatory program governing endogenous $CD8^+$ T cell activation, outlining latent processes that maintain homeostasis of host immune cells.

Tu141. Mechanism of Glucose on Follicular Helper T Cell Activation

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Abstract Text: Intracellular metabolic pathways, particularly glycolysis and oxidative phosphorylation, are key regulators of T cell differentiation and function. Although the mechanism of T cell activation on glucose metabolism has been extensively studied, the reciprocal influence of glycolytic activity on T cell activation remains unclear. Here, we investigated how glucose availability modulates the activation of follicular helper T (Tfh) cells, a subset essential

for B cell maturation, germinal center formation, and high-affinity antibody production. Total Tfh cells or Tfh17 cells, the predominant Tfh subset, were isolated from peripheral blood of healthy donors and stimulated with anti-CD2/3/28 antibodies in complete medium or glucose-free condition. Under glucose deprived conditions, surface expression of activation markers such as ICOS and CD69, as well as cytokines including IL-2, IL-17 and TNF-α in the supernatant, showed reduced or minimal changes at the protein level. RT-qPCR analysis revealed that most transcripts decreased. While secreted levels of IL-2 and TNF-α were low in glucose-free, their transcripts and intracellular protein levels were not decreased, indicating impaired secretion. Furthermore, TCR phosphorylation was reduced under glucose-deprived conditions, regardless of stimulation. This suggests that glucose deprivation affects proximal TCR signaling. These findings reveal that glucose availability exerts multilayered and molecule-specific regulatory effects on Tfh cell activation. Modulation of glucose metabolism may therefore provide a promising approach for fine-tuning Tfh cell function in both physiological and pathological contexts.

Tu142. A Mutable HIV Envelope Model Reveals Immune Constraints on Antigen Diversification

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Abstract Text: HIV transmission and chronic infection result, in part, from rapid viral diversification and immune evasion. These characteristics also undermine fixed-antigen immunization strategies. To explore the mechanisms sustaining diversification and immune evasion, we engineered a murine “mutable transgene” model in which an HIV-1 subtype B envelope (Env) transgene is conditionally expressed under the murine Ig λ light-chain promoter and Ig heavy-chain intronic enhancer that diversifies through B-cell somatic hypermutation. Following immunization with nominal antigen (NP-OVA), activated B cells secrete Env. The enzymatic mechanisms that drive hypermutation cause mutations in the ENV insert to accumulate in germinal center B cells at frequencies comparable to mutations in immunoglobulin genes. Non-synonymous mutations in Env are consistent with those observed in common HIV Env variants. Importantly, Env repertoires evolve in this system in a CD8 T cell–dependent manner, with deletion of B cell clones producing immunogenic peptides and persistence of clone-specific memory. CD8 depletion (i) increased Env mutation frequency per cell and (ii) expanded the repertoires of persistent Env peptides to include peptides with predicted high affinity to host MHC class I. Our findings identify that CD8-dependent responses shape and constrain Env repertoires and affirm that adaptive immunity shapes the evolution of somatically mutating viruses, *in vivo*.

W137. A Reversible Metabolic Checkpoint Controls Human Plasmablast Differentiation and Flu Vaccine Responses Under Immunosuppressive Drug Exposure

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Abstract Text: Patients receiving immunosuppressive medications mount suboptimal vaccine responses, yet how distinct drug classes reshape human germinal center (GC) regulatory programs remains poorly understood. To address this, we used human tonsil-derived immune organoids that recapitulate GC formation, plasmablast differentiation, isotype switching, and influenza-specific antibody production following stimulation with the live attenuated influenza vaccine (LAIV). To define early gene regulatory effects of immunosuppressive drugs, organoids were exposed to commonly prescribed agents spanning multiple drug classes and profiled by single-cell Assay for Transposase-Accessible Chromatin sequencing (scATAC-seq). Drug exposure induced widespread chromatin remodeling across immune cell populations that was both drug and cell-type specific. Antimetabolite drugs, including methotrexate (MTX) and mycophenolate (MPA), produced the most pronounced chromatin remodeling in B cells, characterized by altered accessibility at proliferation-associated enhancers and enrichment of inflammatory and

stress-responsive transcription factor motifs. To determine whether these regulatory changes translated into functional defects during vaccination, we assessed humoral responses following LAIV stimulation. MTX and MPA uniquely suppressed plasmablast differentiation and influenza-specific IgM, IgG, and IgA production, whereas other immunosuppressive classes had more modest effects. Flow cytometry analysis revealed reduced expansion of GC B cells and plasmablasts in a manner independent from cell death. Proliferation tracking demonstrated that antimetabolites impaired B-cell proliferation during GC responses. Despite impaired humoral output, intranuclear flow cytometry showed preserved induction of canonical B-cell transcriptional programs, including downregulation of PAX5 and BACH2 with upregulation of IRF4, indicating intact fate specification. Additionally, suppression was fully reversible upon drug washout or supplementation with folinic acid (MTX) or guanosine (MPA), consistent with a transient metabolic defect. Notably, antimetabolite-treated B cells still retained their ability to support T follicular helper cells and CXCL13 production, suggesting uncoupling of B cell effector function from B-T cell collaboration. Together, these data identify drug and cell-specific chromatin states induced by immunosuppressive medications and define a reversible metabolic checkpoint that constrains proliferation-dependent plasmablast production during human vaccine responses.

W138. Assessing IgM ANA$^+$ B Cell Antibody Reactivity Using Integrated Single-cell Approaches

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Abstract Text: Background: Systemic lupus erythematosus (SLE) is characterized by impaired B-cell tolerance leading to the production of antinuclear antibodies (ANA). Examining ANA-producing B cells at the single cell level can provide important insight into B cell selection in health and disease and the functional properties of ANA in autoimmunity. To address this, we combine a flow cytometry-based assay for the identification of ANA$^+$ B cells with an optimized single-cell (Nojima) culture system that enables clonal expansion and antibody secretion. Because conventional ANA ELISAs assays are optimized for serum samples and are not designed to assess antibodies secreted by single B cells in culture, we developed and validated an ANA ELISA adapted for culture supernatants. Methods: Naïve and IgM$^+$ CD27$^+$ B cells from 2 healthy individuals and patients with lupus were flow-sorted based on ANA binding and ANA$^+$ and ANA- cells were cultured individually using an optimized single-cell culture system with CD40L-expressing feeder cells and BAFF, IL-2, and IL-21. By day 26, most cultures produced >1 µg/mL immunoglobulin. ANA reactivity of supernatants was evaluated by ANA ELISA and compared between ANA$^+$ and ANA$^-$ populations. Results: In naïve IgM B cells, the assay demonstrated a specificity of 86% and sensitivity of 78%. In IgM CD27$^+$ B cells specificity and sensitivity were 70% and 68% respectively. Conclusion: Together, these findings establish a single cell platform for the generation of ANA$^+$ IgM B cells. This approach provides a foundation for dissecting early B cell tolerance checkpoints and for future antigen specific profiling in SLE.

W139. NK Cell Regulation Constrains B Cell Immunity to Therapeutic Vaccination in Chronic HBV Infection

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Abstract Text: Chronic HBV infection (CHB) represents a significant healthcare burden worldwide, for which new immunotherapeutic strategies to achieve sustained, viral control off-treatment (functional cure) are urgently needed. Therapeutic vaccination can engage antigen-specific immune responses to HBV, but effectiveness is hampered by dysfunctional T and B cell responses. We previously demonstrated that NK cells negatively regulate CD8 T cell responses to therapeutic vaccination in CHB; emerging data in other infection and vaccination settings suggest they also inhibit neutralising antibody production. We are therefore examining whether manipulating NK cell regulation

can enhance humoral immunity to achieve anti-HBs seroconversion, a hallmark of functional cure in HBV. NK cell depletion prior to ChAdOx-HBV therapeutic vaccination significantly enhanced expansion of splenic follicular helper T cells (Tfh) and germinal centre (GC) B cells in adeno-HBV infected mice. In highly viraemic AAV-HBV infected mice vaccinated with HBsAg and HBcAg (analogous to the TheraVacB regimen), NK cell depletion similarly boosted Tfh, GC B cells and anti-HBs production. Circulating Tfh, expressing key effector molecules (CD40L), from donors with CHB were selectively eliminated by autologous NK cells during cocultures. B cells from patients with CHB could be rescued to produce anti-HBs by coculturing with autologous *in vitro* differentiated Tfh, which were in turn eliminated by NK cells. In human lymphoid organoid models, NK depletion led to greater plasmablast expansion in response to HBV therapeutic vaccination. Through mining scRNA-seq data of human vaccine responses we explore tractable pathways that underpin this negative NK cell regulation to enhance anti-HBs seroconversion following therapeutic vaccination.

Basic Science of Immunology - Innate Immunity

Th145. Innate Immune Modulation by Staphylococcal Superantigens: Divergent Effects on NK and γδ T Cells

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Abstract Text: Staphylococcal enterotoxins, also known as superantigens (SAgs), are well characterized for their effects on αβ T cells. In contrast, their impact on the innate immune system remains poorly understood. Emerging evidence suggests that these toxins may disrupt early immune responses, favoring bacterial dissemination and immune dysregulation, leading to effector cell depletion and contributing to autoimmunity, hypersensitivity, and tumor development. To investigate the impact of SAgs on innate cytotoxic cells involved in pathogen control and immune surveillance, we analyzed the effects of recombinant egc-encoded SAgs (SEG, SEI, SEM, and SEO) on isolated human NK cells and γδ T cells from PBMCs, as well as on an NK cell line. SEG and SEM significantly enhanced NK cell-mediated cytotoxicity against the K562 target cell line ($p < 0.01$ vs untreated), whereas SEO inhibited this activity. In γδ T cells, SEG, SEM, and SEO induced activation, as reflected by increased CD69 expression in a dose-dependent manner (SEG $p < 0.05$, SEM $p < 0.001$, SEO $p < 0.01$), and promoted a pro-inflammatory response characterized by IFN-γ and TNF-α production. In addition, SEG and SEO induced apoptosis of stimulated γδ T cells (SEG $p < 0.0001$, SEO $p < 0.05$). Interestingly, these effects on γδ T cells were not dependent on the canonical αβ TCR binding site, since N24A-SEG, a mutant impaired in αβ TCR interaction, induced cytokine secretion at levels comparable to wild-type SEG. In contrast, SEI did not significantly affect NK cell cytotoxicity or γδ T cell activation. Together, these findings highlight the functional versatility of egc-encoded superantigens in modulating distinct innate cytotoxic populations, promoting inflammatory responses while simultaneously inducing immune suppression. Our results suggest that SAgs may use different structural surfaces or “hot spots” to target distinct immune cell types, and that some of their activities may require cell–cell interactions.

Th146. Blocking of PD-1 and FABP4 Rescues NK Cell Function: Relevance in Psychosocial Stress and Cardiovascular Disease Risk

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Abstract Text: Background: The physiological impact of chronic stress can be driven by cortisol-mediated signaling, influencing the distribution and function of Natural Killer (NK) cells. The underlying mechanisms connecting psychosocial stressors, cortisol, and NK cells need to be deciphered. Methods: We recruited 186 African American (AA) women from the Step It Up Community-Engaged, Digital Health Physical Activity Intervention. Psychosocial stressors (depression, chronic stress, social isolation, and loneliness) were determined using validated questionnaires. Multivariable linear regression was applied to investigate associations between cortisol, NK cells, and psychosocial stressors. All models were adjusted for age, SES, and cardiovascular risk factors. Additionally, using our data from an obesity-based study, the RNA sequencing dataset was reanalyzed based on participants' plasma cortisol levels. NK cell function was assessed by blocking key upregulated genes (PD-1, PD-L1 & FABP4) of the cortisol-NK cell pathway. Immunophenotyping and functional analysis of NK cells were done by flow cytometry. Results: We observed a negative association between cortisol and proliferative NK cells (β=-0.21, p=0.006). Cortisol is known to inhibit NK cells, as also demonstrated by our *in vitro* study, which showed cortisol-mediated inhibition of primary NK cells. Reanalyzing the obesity-lean RNA sequencing dataset by comparing high versus low plasma cortisol levels identified genes, which, when overlapped with upregulated NK cell genes in a published human atherosclerotic patients' dataset, allowed us to identify PD-1 and PD-L1 as key upregulated genes. Blocking PD-1 in cortisol-treated NK cells partially rescued cytolytic function (as measured by CD107a) but not cytokine production (IFN-γ & TNFα). FABP4 inhibition fully rescued cortisol-induced PD-1 upregulation, restoring levels to those of untreated controls. FABP4 inhibition also rescued cortisol-mediated reductions in NK cell degranulation, highlighting FABP4 as a key mediator of stress hormone-driven NK cell dysfunction. Conclusion: FABP4 is one of the major upregulated proteins observed among individuals experiencing loneliness. FABP4 upregulation and the glucocorticoid-mediated increase in PD-1 levels, especially in the context of chronic stress and loneliness, suggest a psychosocial–neuroendocrine–immune pathway that could contribute to increased cardiometabolic risk. Physical activity interventions may be an approach to lower elevated levels of cortisol among individuals experiencing chronic stress and loneliness.

Tu143. Characterization of Innate Immune Responses and Severity Biomarkers in Neonatal Sepsis in India

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Abstract Text: Sepsis is caused by dysregulated innate immune response to infection, leading to organ dysfunction and high mortality. India bears the highest global burden of neonatal sepsis, with fatality rates reaching 65%. To address this critical challenge, the Government of India has launched a multi-disciplinary and multi-institutional program to develop solutions for neonatal sepsis. As part of this initiative, our study focuses on delineating innate immune responses in preterm neonates, a highly vulnerable group. Aberrant production of immune mediators may impair pathogen recognition and clearance, contributing to severe outcomes, yet factors determining why some neonates recover while others deteriorate remain poorly understood. The objectives of this study are to characterize dysregulation in innate immune responses and to identify immune phenotypes associated with clinical outcomes in sepsis-suspected neonates. We have collected peripheral blood samples from over 300 such neonates and analyzed monocytes and their classical, intermediate, and non-classical subsets using multiparametric flow cytometry. Our findings reveal a significant reduction in non-classical monocytes. Although HLA-DR expression was

downregulated, consistent with existing literature, it did not differentiate between recovered and deceased neonates. Notably, classical monocytes exhibited marked downregulation of the chemokine receptors CX3CR1 and CCR2, essential for homing, patrolling, and extravasation, suggesting potential functional impairment. We are now applying machine learning approaches to identify expression patterns that may serve as early predictors of adverse outcomes. This work aims to identify innate immune markers that enable timely risk stratification and improved clinical management of neonatal sepsis while advancing understanding of early-life immune determinants of survival.

Tu144. CX-01 Mitigates Acute Kidney Injury and Improves Survival in a Rat Polytrauma Model

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Abstract Text: Severe polytrauma can induce devastating complications, including acute kidney injury (AKI) and other organ failure. If left untreated, these types of complications have been shown to lead to high mortality. The pathophysiological mechanisms underlying organ dysfunction remain inconclusive, however, previous studies have shown that the body's innate immune response may contribute to trauma-induced organ failure. The objective of this study was to test whether treatment with CX-01, a high mobility group box 1 (HMGB1) inhibitor, will reduce AKI and improve survival in a rat model. Male Sprague Dawley rats were anesthetized and given proper analgesics, underwent polytrauma (soft tissue injury, fibula facture and hemorrhage), received blood resuscitation to maintain a MAP of 90 mmHg, and then monitored for 72 hours or upon reaching a humane endpoint. The animals were randomly assigned to either injury control (polytrauma, n=9), or therapeutic treatment (polytrauma+CX-01, n=8). The vital signs, blood chemistry, inflammation, and tissue damage were recorded and analyzed at baseline and post-injury. 3 out of 9 injured control animals survived to the end of the study (mortality=67%), while 7 out of 8 animals in the CX-01 treatment group survived (mortality=13%, $p< 0.05$). Use of CX-01 significantly improved glomerular filtration rate from 0.56±0.07 to 0.84±0.33 at end of resuscitation ($p< 0.05$). CX-01 treatment reduced HMGB1 levels, inflammatory cytokines (MCP-1, RANTES, IL-10 etc.), and attenuated kidney damage observed by gross pathology. CX-01, as an immunotherapeutic, mitigates trauma-induced immune response, AKI and improves survival. CX-01 may serve as an effective therapeutic to reduce trauma-induced AKI in combat casualties.

Tu145. A Proliferative IL-4–programmed Macrophage Axis Predicts Impaired Reverse Remodeling Following LVAD Implantation

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Abstract Text: Objectives: To establish *in vitro* conditions to model myocardial macrophage programming in the heart tissue of patients treated with left ventricular assist devices (LVAD). To assess the predictive value of macrophages for cardiac recovery under LVAD therapy. Methods: Primary human $CD14^+$ monocytes were polarised with IL-4 (±TGF-β) for 36 days and profiled by bulk RNA-seq and phosphoproteomics. An IL-4–36d gene signature was scored across macrophage states in a public LVAD heart snRNA-seq dataset. Multi-omics candidates were quantified by multiplex immunofluorescence alongside CD68, stabilin-1 and Ki-67 in paired pre-LVAD biopsies and post-LVAD explants from 22 patients. LVAD response was defined a priori using a composite left ventricular ejection fraction (LVEF) + left ventricular end-diastolic diameter (LVEDD) criterion (responders vs non-responders), and associations with ΔLVEF/ΔLVEDD were tested using logistic and mixed-effects regression. Results: Prolonged stimulation of human primary macrophages with IL-4, but not IL-4+TGF-β, maintained viability and induced a $CD68^+$ multinucleated macrophage state with inflammatory, ECM-remodelling and proliferative transcriptional modules. Phosphoproteomics demonstrated activation of cell-cycle regulators at day 36. In LVAD snRNA-seq, biomarkers of IL-4–treated macrophages at day 36 were enriched in discrete myocardial macrophage states, linking the *in vitro*

model to myocardial macrophages. In paired samples, proliferative macrophage subsets were differentially abundant at baseline: Ki-67$^+$CD68$^+$ and Ki-67$^+$pSer378$^+$CD68$^+$ macrophages were enriched in non-responders. Across patients, baseline Ki-67 burden inversely correlated with ΔLVEF (Spearman ρ=−0.51, P=0.014). Adding Ki-67 burden to baseline LVEF/LVEDD improved discrimination for response (AUC increased from 0.52 to 0.83; optimism-corrected AUC 0.74). Longitudinally, mixed-effects modelling showed divergent immune remodelling trajectories: Ki-67$^+$ and pSer378$^+$ macrophage subsets expanded post-LVAD in non-responders, consistent with maladaptive persistence of a chronic IL-4 pathway. Conclusions: Chronic IL-4–induced programming persists in LVAD myocardium and defines a proliferative macrophage axis that is associated with reduced reverse remodelling and non-response to LVAD therapy.

W140. Elucidating Molecular Mechanisms of Natural Killer (NK) Cell Function Through Studies of NK Cell Desensitization

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Abstract Text: Natural killer (NK) cells play a critical role in immune surveillance by eliminating tumor cells that evade CD8$^+$ T cell-mediated control, including those that lose expression of self-MHC class I molecules. However, persistent exposure to activating or MHC class I-deficient environments can progressively impair NK cell responsiveness, a process often referred to as NK cell desensitization. The molecular mechanisms that enforce this dysfunctional state remain poorly defined, limiting our ability to harness NK cells therapeutically. To identify cell-intrinsic regulators of NK cell desensitization, we systematically compared transcriptomic profiles of functional and desensitized NK cells across multiple tumor-free models of persistent stimulation. This integrative approach revealed a set of shared gene expression changes despite substantial contextual diversity, highlighting signaling regulators that may act as conserved brakes on NK cell function. Among these, the inositol kinase ITPKB emerged as a prominent candidate. Functional and mechanistic analyses demonstrated that genetic or pharmacologic suppression of ITPKB in mature murine and human NK cells restored degranulation and cytotoxicity, particularly under desensitized conditions. These effects were linked to improved calcium signaling downstream of activating receptors. Importantly, pharmacological inhibition of ITPKB enhanced NK cell-mediated tumor control *in vivo* by reinvigorating the functional capacity of desensitized tumor-infiltrating NK cells. Moreover, genetic deletion of ITPKB improved the efficacy of adoptively transferred CAR-NK cells, underscoring its translational potential. Together, these studies establish NK cell desensitization as an actively regulated state and provide a transcriptomic resource that highlights additional candidate regulators of NK cell dysfunction for future mechanistic and therapeutic exploration. Furthermore, by combining systematic transcriptomic profiling with targeted perturbation, this work identifies actionable molecular pathways that constrain NK cell effector function and provides a framework for developing next-generation NK cell-based immunotherapies.

W141. Microneedle-mediated Local Delivery of the TLR7 Agonist Imiquimod in Human-analog Skin: A Platform for Controlled Cutaneous Innate Immune Modulation

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Abstract Text: Background: Imiquimod is a Toll-like receptor 7 (TLR7) agonist widely used to stimulate innate immune responses in cutaneous disease. However, conventional topical application is limited by variable skin penetration, inconsistent localisation, and potential off-target exposure. There is a need for delivery platforms that enable controlled, localised exposure of innate immune agonists within immune-relevant skin layers to support translational cutaneous immunology research. Methods: We developed dissolvable sugar-based microneedle arrays loaded with imiquimod and evaluated their delivery performance using an in-vitro porcine ear skin model, a validated

human skin analogue. Microneedle insertion, dissolution, and compound deposition were assessed histologically to determine penetration depth, tissue integrity, and localisation within epidermal and superficial dermal compartments relevant to innate immune sensing. Results: Microneedle arrays consistently penetrated the stratum corneum and dissolved within the skin, enabling reproducible local deposition of imiquimod across targeted cutaneous layers. Histological assessment demonstrated preservation of overall skin architecture following microneedle application, with no evidence of gross tissue disruption. Delivery was spatially confined to immune-relevant skin compartments, supporting the feasibility of microneedle-facilitated localisation of TLR7 agonists. While downstream immune activation was not quantified in this study, the platform reliably achieved controlled cutaneous delivery suitable for subsequent immune-phenotyping investigations. Conclusion: This study establishes microneedle-mediated delivery as a robust platform for the localised administration of innate immune agonists in human-analog skin. By enabling controlled exposure of TLR7 agonists within defined cutaneous compartments, this approach provides a scalable experimental framework for future studies investigating skin-resident innate immune responses and translational cutaneous immunomodulation.

W142. Rethinking Gene Expression Analysis Through the Lens of Conservation

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Abstract Text: Mus musculus is a model organism commonly used in biomedical research, and many basic immunologic principles were discovered through mouse studies. However, findings from mouse models often do not translate into human clinical settings. The challenge of predicting which discoveries in mouse experiments are reproducible in human trials or determining what aspects of a human disease can be recapitulated in a mouse model continues to thwart basic and translational scientists. The most recent common ancestor between the rodent and primate lineage was around 80 million years ago, and much of their genomes have diverged since then. When considering whether a gene has species-specific or conserved expression, the nucleotide sequence of the gene is compared with its mouse or human counterpart(s). However, structural variations also play substantial roles in altering the expression patterns of genes as they can alter the genomic environment surrounding genes. We established an analysis strategy to discern which genes contribute to differences or similarities in transcriptional profiles between mouse and human immune cells. Specifically, we analyzed 10 independent RNA-seq datasets (5 mice and 5 human) from LPS inducible macrophage experiments. Using gene ortholog and expression data, we performed comparative analysis between mouse and human macrophages. Out of all the DEGs, only 40.0% of human genes and 26.7% of mouse genes possessed comparable transcriptional profiles across species. We further performed gene set enrichment analysis (GSEA) on varying groups of DEGs delineated by orthologous relation and/or expression profile. Results from the canonical GSEA aligned more closely with results from genes with comparable expression profile across species. Notably, pathways related to chemotaxis were highly enriched in species-specific genes. Finally, we developed an approach to visualize and quantify the diverging genomic structures between the mouse and human genomes using gene location, and over 500 evolutionary breakpoints were identified. We are developing a novel and user-friendly conservation analysis tool to better inform whether a DEG will maintain the same genomic landscape in the other species. Our approach to interweave conservation with gene expression and pathway analysis demonstrates the possibility of narrowing down gene targets for translational research and mouse model design.

W143. IRAK4 Degrader Combines Degradation and Kinase Inhibition to Achieve Functional Effect

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Abstract Text: Aberrant activation of the immune system through toll-like receptors (TLRs) and pro-inflammatory cytokines drives the pathogenesis of multiple autoimmune and autoinflammatory diseases. Most TLRs and the IL-1 receptor (IL-1R) cytokine family signal via the Myddosome, a multiprotein complex in which IRAK4 (interleukin-1 receptor-associated kinase 4) is an essential component. Pre-clinical and clinical data suggest that suppressing IRAK4 activity is efficacious in treating multiple inflammatory diseases, such as rheumatoid arthritis and atopic dermatitis. In addition to kinase activity, IRAK4 has a scaffolding role in the Myddosome. To disrupt both functions, we leveraged the ligand-directed degradation (LDD) approach: bifunctional small molecule degraders with a kinase inhibitor warhead were designed to selectively target IRAK4 for proteasomal degradation, as well as inhibit its enzymatic function. This dual mechanism of action is expected to achieve robust and sustained inhibition of TLR/IL-1R-mediated inflammatory signaling. Our IRAK4 LDD is potent, selective, and orally available, achieving 90-99% degradation of IRAK4 *in vitro* and *in vivo* across multiple species. A single oral dose of IRAK4 LDD resulted in >14-day suppression of IRAK4 levels in cynomolgus macaque peripheral blood mononuclear cells (PBMCs). Across *in vitro*, *ex vivo* and *in vivo* models, IRAK4 degradation enhanced cytokine inhibition compared to kinase inhibition alone under TLR4-driven and IL-1R-driven stimulation. However, under TLR7/8 stimulation, kinase inhibition was the dominant mechanism of action, indicating that the relative contributions of IRAK4's kinase and scaffolding functions are context-dependent.

Immuno-engineering and Cellular Therapies

Th147. A Treg Cell-targeted IL-2m Results in Superior Suppression of Th2 Responses Compared to CD25-biased IL-2m and anti-IL4R Blockade in a Mouse Model of Asthma

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Abstract Text: IL-4R blocking approaches are efficacious in atopic dermatitis but rebound is often observed after stopping treatment due to persistence of disease-driving type 2 cells. IL-2 based therapies, including IL-2 muteins and pegylated IL-2, have been engineered to increase Treg cell activity and have shown clinical promise in atopic dermatitis. These CD25-biased IL-2 approaches are typically engineered to reduce affinity to IL-2Rb to drive reliance on CD25 for preferential activity on Treg cells. However, CD25 is transiently expressed upon Tconv activation and constitutively expressed on human Th2s and ILC2s. We confirmed that human and mouse (Fc.mut24) IL-2m expand Tregs *in vivo* but also increased ILC2s and eosinophils frequencies as well as increased type II cytokines IL-5/IL-13 in serum. When mice were treated therapeutically in a ova/alum Th2-driven airway inflammation model, neither IL-2m nor anti-IL-4R reduced Th2 frequencies. We hypothesized that a Treg cell-targeted IL-2m would lead to a deeper reduction in Th2 responses. We generated a transgenic model, FOXP3-Cre x LSLhumanCD4 (FOXP3hCD4) for FOXP3-driven expression of human CD4 along with a hCD4-IL-2m molecule consisting of a human CD4 targeting Fab clone (lacking mouse cross reactivity) and an IL-2m with attenuated affinity to IL2Rb and IL2Ra (Treg-IL-2m). Treg-IL-2m expanded Treg cells in both periphery and tissues without ILC2 increase or serum IL-5/IL-13

upregulation. When dosed prior to challenge, Treg-IL-2m exhibited superior suppression of the numbers and frequencies IL5^{+}IL-13^{+} Th2 cells and increase in Treg cells within the lung/BALF compared to CD25-biased IL-2m and anti-IL-4R. Finally, we assessed potential for drug free remission with anti-IL-4R and Treg-IL-2m combination and demonstrated sustained off-therapy reduction in Th2 and anti-Ova IgE responses compared to either single agent alone. Taken together, these results highlight the potential of a Treg cell-targeted IL-2m for superior suppression and restoration of immune tolerance in Th2-driven indications.

Th148. Engineered CD4LVFOXP3 Treg Cells in the Clinic: Interim Data in Patients with Monogenic Autoimmune IPEX Syndrome

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Abstract Text: The Orphan Drug product, engineered CD4LVFOXP3 regulatory T (Treg) cells, is obtained from CD4^{+} T cells lentiviral vector (LV)-transduced with EF1-FOXP3; phenotypically and functionally resemble naturally occurring Treg, and express the membrane marker tNGFR (CD271). We have developed a First-In-Human (FIH) Phase 1 clinical trial with this novel Treg cell therapy product to treat IPEX syndrome (NCT05241444), a life-threatening monogenic autoimmune disorder classically associated with enteropathy, early-onset diabetes, and severe atopic dermatitis. The protocol design is a 2^{+}4 dose escalation in two different age cohorts, ≥12 years (A) and < 12 years of age (B). Nine patients have been treated with the high-purity product. Primary endpoints of feasibility and safety have been successfully achieved with the lowest (DL1, 1x106/kg; n=2), intermediate (DL2, 3x106/kg; n=2), and highest (DL3, 10x106/kg; n=2) doses in the older patients, and in three of the younger patients at the DL2 (n=2) and DL3 (n=1) cell doses. There have been no significant or related adverse events. In nearly all patients, we observed a notable clinical response, including significant improvement in growth, gastrointestinal symptoms and disease biomarkers after treatment. Following infusion, CD4LVFOXP3 cells are detectable in the peripheral blood, exhibiting dose-dependent levels and duration of persistence, and the ability to migrate to the tissues. CD4LVFOXP3 Treg cells demonstrate phenotypic and functional stability at the single-cell level by CITEseq performed on the product preinfusion and *in vivo*. CD4LVFOXP3 maintains the Thelper subsets' heterogeneity of the parental cells, TCR polyclonality, including autoreactivity, and expresses the transgene FOXP3-lv, and the expected Treg markers. CD4LVFOXP3 contains a high proportion of cells with T-stem cell memory features, derived from naïve CD4^{+} T cells, suggesting that they acquired high survival potential. After infusion, the Treg features are maintained and the patient's peripheral blood samples exhibit lower expression of pro-inflammatory cytokines. CD4LVFOXP3products are safe, preserve their Treg signatures, and persist *in vivo*, with evidence of clinical benefit. Successful completion of this Phase 1 trial will address a significant unmet medical need while providing proof of safety and feasibility of this novel therapy. These data could expand the application of CD4LVFOXP3 to additional autoimmune disorders.

Th150. Development of Tri-specific Extracellular Vesicles for Anti-cancer Therapeutics

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Abstract Text: The clinical integration of bispecific T cell engagers (BiTEs) such as bispecific antibodies (bsAbs) have been widely adopted into treatment regimens for several cancer subtypes, however, their efficacy remains hindered by weak immune synapse formation and off-target effects that manifest as dose-limiting toxicities. We previously leveraged naturally occurring biocompatible messengers, Red Blood Cell Extracellular Vesicles (RBCEVs), to develop a highly customizable nano-engager platform. These Bispecific Extracellular Vesicles (BEVs) are surface-engineered to simultaneously engage CD3 for T Cell activation and Claudin 18.2 on pancreatic cancer cells for precise tumour localization. While BEVs demonstrated enhanced T Cell infiltration, superior tumour regression, and prolonged anti-tumour immunity compared to free antibodies *in vivo*, their overall clinical applicability is challenged by tumour heterogeneity and evolution. To overcome the reliance on a single tumour antigen and mitigate immune evasion via antigen downregulation, we developed a next-generation tri-specific RBCEV (TEV) nano-engager for pancreatic cancer treatment. Utilizing click-chemistry, we decorated RBCEVs with antibodies against two distinct tumour antigens—EpCAM and Claudin 18.2—alongside the T Cell activation receptor CD3. We optimized this tri-specific configuration by screening various antibody stoichiometries, using epitope neutralization and cytotoxicity assays to identify the ideal balance for T Cell recruitment and cytotoxic potency. Our findings demonstrate that TEVs effectively direct T cells to heterogeneous cancer populations, maintaining therapeutic efficacy despite varying antigen levels. Systematic screening revealed that precise relative ratios are critical for balancing immune activation and functional potency to minimise the risk of excessive off-target toxicity. Under optimized conditions, TEVs triggered robust, dose-dependent T Cell activation and superior tumour cell elimination compared to bispecific counterparts in models of antigen heterogeneity. This study establishes tri-specific RBCEVs as a novel, programmable, and precise iteration of immune-engaging technologies capable of countering tumour evolution in refractory malignancies.

Th151. QEL-005: A CD19 Directed CAR Regulatory T Cell Therapy for Autoimmune Diseases Characterized by Multicellular Immune Dysregulation

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Abstract Text: Immune-mediated inflammatory diseases, such as rheumatoid arthritis (RA) and systemic sclerosis (SSc), are multifactorial disorders characterized by dysregulated immune activation involving diverse pathways. Pathogenesis includes immune cell infiltration, autoantibody production, and the acquisition of antigen-presenting capacities by B cells, accompanied by excessive cytokine secretion by T cells that sustains a chronic inflammatory milieu. Under physiological conditions, macrophages maintain tissue homeostasis through clearance of apoptotic cells and support of tissue repair; however, in chronic disease, persistent activation by inflammatory cytokines and cellular debris drives tissue injury and fibrosis. Regulatory T cells (Tregs) are pivotal regulators of immune and tissue equilibrium, exerting control of multiple immune subsets through diverse mechanisms. We propose that Tregs targeted to sites of inflammation could attenuate the pathogenic activity of a variety of cell types including B cells, T cells, and macrophages, thereby mitigating inflammation and facilitating a restoration of tissue homeostasis. QEL-005 is a CD19-specific chimeric antigen receptor (CAR) Treg therapy incorporating a FOXP3 phenotype lock cassette, designed to stabilize Treg lineage and function while enabling antigen dependent activation in B cell–rich lymphoid and tissue niches. Objectives: Determine if CD19 engagement by QEL-005 can modulate B cells and additional immune subsets, including T cells and macrophages via bystander suppression. Methods: A FOXP3 phenotype lock cassette and a CD19 single chain variable fragment (scFv) CAR was introduced into Tregs and expanded for 14 days, cryopreserved, and analyzed to confirm Treg identity. Functional activity was examined in

coculture assays with primary human B cells, T cells, and fully differentiated mature macrophages. Results: QEL-005 exhibited a stable Treg phenotype without detectable cytotoxicity toward B cells. In B cell cocultures, QEL-005—but not untransduced Tregs—reduced CD80/CD86 expression, suppressed antibody production, and limited plasma cell differentiation. In cocultures, QEL-005 induced antigen specific inhibition of T cell proliferation and modulated IFNγ stimulated macrophages toward a more pro-resolving phenotype, characterized by elevated IL-10 secretion and increased matrix metalloproteinase activity. Conclusion: QEL-005 CD19 CAR-Tregs modulate several dysregulated cell types implicated in RA and SSc, demonstrating potential to re-establish tissue homeostasis in these diseases and in a broad spectrum of complex autoimmune diseases.

Th152. Engineering Islet-specific Synthetic Receptors by Modulating Signal Transduction Can Generate a Treg Cell Therapy for Type 1 Diabetes

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Abstract Text: Efficacy of Treg therapy for type 1 diabetes in preclinical models depends on local activation of Tregs within the islets mediated by islet antigen-specific TCR and CD28-mediated co-stimulation. Identifying potent β cell-specific T cell receptors (TCRs) capable of responding to naturally presented human islet antigens remains a major challenge. To develop a Treg therapy for T1D, we selected the 1E6 TCR, which recognizes an abundantly presented, β cell-specific preproinsulin peptide. However, 1E6 exhibits low affinity (Kd of ~300 μM) for its cognate peptide. To overcome this limitation, we performed CDR3-directed mutagenesis to affinity-mature this TCR. The leading variant, TWG, displayed a ~5 × 10^4-fold lower activation threshold than 1E6 in $CD8^+$ T cells. Despite this marked increase in reactivity, conventional $CD4^+$ (Tconv) cells bearing the TWG TCR failed to activate even at high peptide concentrations (up to 100μM), indicating this TCR remained dependent on the CD8 co-receptor. A major function of a co-receptor is to recruit Lck to the TCR to initiate signal transduction. We hypothesized that physically linking TWG to Lck-binding domains from CD8 or CD4 may overcome the co-receptor dependence. Fusion of the TCRβ chain with the Lck-binding domain, "CxCP", increased TWG activation in $CD4^+$ Tconv cells at low peptide concentrations (~0.1-1 μM). Using these novel TCR fusions (referred to as TWGLBD), we show that the Lck binding site is necessary and sufficient for initiating HLA-class I-restricted TCR activation in $CD4^+$ Tconv cells. Altogether our data shows that engineering an Lck-binding site into TCRs can bypass co-receptor requirement in T cells. Tregs depend on co-stimulatory signals for activation and proliferation, however pancreatic β cells do not express co-stimulatory ligands. We thus generated an islet-specific chimeric co-stimulatory receptor (COSTR) by fusing an islet-specific DPP6 nanobody with an IgG hinge and CD28 intracellular signaling domains. Finally, co-expressing TWGLBD and COSTR in Tregs allowed for robust activation and proliferation when co-cultured with human β cells. These β cell-specific Tregs effectively suppressed activation and proliferation of autoreactive T cells *in vitro*. These results demonstrate that rationally designed synthetic β cell-specific receptors can support Treg activation and suppression by endogenous β cell antigens.

Th153. Non-ICANS Neurotoxicity Following BCMA CAR T Cell Therapy: Distinguishing Movement and Neurocognitive Toxicity from Peripheral Nervous System Manifestations

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Abstract Text: Objectives: CAR-T cell therapy (CAR-T) has transformed the treatment of hematologic cancers and autoimmune diseases. With improved control of acute toxicities, long-term immune-mediated complications have gained clinical relevance. Here, we characterize clinical phenotypes and underlying immune mechanisms in patients with non-Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) neurotoxicity following BCMA-directed CAR-T. Methods: We prospectively enrolled patients with Movement and Neurocognitive Toxicity (MNT) or neuropathy (NEU) following CAR-T with ciltacabtagene autoleucel (cilta-cel) for multiple myeloma. Clinical characteristics, outcomes, laboratory analyses, and Olink® proteomics in blood and cerebrospinal fluid (CSF) were compared between the groups. Results: Of 148 patients receiving CAR-T in our timeframe, 21 developed non-ICANS neurotoxicity (14.2%), including 8 with MNT (median age, 65 [IQR: 60-72], 6 males) and 13 with NEU (median age, 63 [IQR: 61-72]; 11 males; 6 cranial neuropathies, 1 peripheral neuropathy, 5 combined). Time from CAR-T to onset was 27 and 24 days for MNT and NEU, respectively (p=0.827). MRI revealed basal ganglia T2/FLAIR hyperintensities in only 2/8 MNT cases, whereas nerve enhancement was notable in 6/12 patients with NEU. In contrast, CSF pleocytosis, often with $CD4^+$ CAR predominance, was common in MNT but not NEU (median CSF cell count, 182/µL vs. 1/µL, p=0.035). Patients with MNT had higher baseline LDH levels (p=0.039) and 30-day peak absolute lymphocyte counts (p=0.005), suggesting worse myeloma disease and CAR expansion. In MNT, serum proteomic pathway analysis suggested upregulated cellular proliferation, while the CSF proteome highlighted T cell receptor signaling, NK activation, and apoptosis with pronounced CNS injury. In NEU, proteomic pathways related to IL-6-mediated JAK-STAT signaling were particularly upregulated in the serum. Corticosteroids, high-dose IVIg, and second-line immunotherapy were administered in 6/8 vs 13/13, 4/8 vs 6/13, and 3/8 vs 1/13 patients with MNT and NEU, respectively. Symptom reversibility was unfavorable in MNT compared to NEU: All MNT patients experienced some sequelae, while 9/13 patients with NEU achieved complete recovery (p=0.002). Conclusion: One in seven patients developed non-ICANS neurotoxicity after cilta-cel. MNT appears as a partially irreversible T cell-driven phenotype, whereas NEU involves systemic IL-6 pathways with reversible outcomes. Elevated baseline LDH and increased CAR expansion may predispose for MNT.

Th154. PD-1^{high} and CD39 Dual Selection Strategy for the Generation of Tumor-Infiltrating Lymphocyte Therapy in Lung Adenocarcinoma

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Abstract Text: Adoptive cell therapy based on tumor-infiltrating lymphocytes (ACT-TILs) has shown promising results in melanoma, yet its efficacy in other solid tumors, such as lung adenocarcinoma, remains limited. This may stem from the low frequency of naturally occurring tumor-reactive T Cells or from the loss of specific clones during the expansion process. To overcome this, we need strategies that enrich tumor-reactive T Cells directly from the tumor. One approach is to select TILs based on the expression of biomarkers of tumor-reactivity. PD-1, CD39 or CD137 have emerged as strong candidates. In this study, we propose a dual selection strategy based on PD-1^{high} and $CD39^+$ expression to enrich TIL products in tumor-reactive clonotypes, reducing bystander expansion, and preserving functional attributes compared with PD-1 selection alone. We first analyzed biopsies from different solid tumor subtypes, including lung adenocarcinoma, and characterized PD-1, CD39, CD103, and CD137 expression by

flow cytometry and the secretome by single cell analysis. Our data revealed that CD39, either alone or combined with PD-1, identifies a broader subset of activated T cells than PD-1 alone. Based on these findings, we established a dual selection protocol. TILs, from fresh lung adenocarcinoma biopsies, were sorted using the MACSQuant® Tyto® system into PD-1^{high} and PD-1^{high} CD39^{+} fractions and expanded with allogeneic feeders, anti-CD3, anti-CD28, and IL-2 (3000 U/ml) until reaching 50 million cells. Both fractions achieved comparable yield, viability, and immunophenotype after expansion. We then validated an in-house multiplex PCR to amplify TRB CDR3 from genomic DNA and performed NGS analysis. Dual selection reduced TRB CDR3aa repertoire diversity and increased clonality compared to PD-1^{high}, with distinct clonotypes expanded by fraction. TRB CDR3aa sequences were annotated using public databases and ImmuneWatch DETECT®. Autologous tumor cell lines are being generated to compare the functionality of PD-1 ^{high} CD39^{+} TILs versus PD-1 ^{high} TILs. Further analysis will be needed to demonstrate higher specificity. Also, TILs will be undergone Rapid Expansion Protocol (REP) and similar analysis will be performed. This strategy could improve the specificity of TIL therapy and provide a pipeline for identifying tumor-specific TCRs for next-generation transgenic TCR approaches.

Th155. Dynamics of T Cell Engager-mediated T Cell Activation and B Cell Killing in Patient-derived Coculture Systems

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Abstract Text: T cell engagers (TCE) are emerging as a promising therapeutic modality for deep B cell depletion in immune-mediated diseases. Multiple studies with different TCEs have demonstrated rapid B cell depletion and a concurrent improvement of clinical disease activity in patients with a range of severe, multidrug-resistant autoimmune diseases. However, the dynamics of T cell activation, cytokine release, and B cell killing, as well as implications on safety in patients with autoimmune diseases are still poorly understood. Here, we established a coculture system with total PBMCs from healthy donors and patients with rheumatoid arthritis. Different concentrations of the CD3xCD19 TCE blinatumomab were employed, ranging from 1 to 100 ng/ml. Cell populations were assessed at multiple timepoints from 0 to 72 hours via flow cytometry and cytokines were quantified via multiplex bead assay. The model accurately depicted a dose-dependent decrease in B cell frequency, with an earlier and more pronounced B cell depletion in high blinatumomab concentrations. Over time, we simultaneously observed a dose-dependent downregulation of CD19 surface expression on the remaining CD19^{+} B cells. TCE-mediated killing led to an increased expression of several activation markers on T cells including PD-1, LAG-3, TIGIT, and TIM-3, as well as to changes of subset frequency within the CD4^{+} and CD8^{+} T cell compartments. Further, we detected a dose- and time-dependent elevation of granzyme B, perforin, and IFN-γ in the supernatant. To investigate the contribution of different T cell subsets to the overall B cell depletion efficiency, we subsequently established coculture assays of B cells with different purified T cell subsets. In these experiments, killing capacity was heterogenous: CD8^{+} T cells, and particularly CD8^{+} T memory cells, were the most potent effectors. These analyses further revealed distinct differences between the expression level of activation markers and the amount of cytokine in the supernatant among the T cell subsets. Taken together, this study provides key mechanistic insights underpinning the feasibility of TCE therapy in patients with autoimmune disease and contributes to a better understanding of biomarkers that can be leveraged to optimize safety and clinical efficacy.

Th157. From Mutations to Targets: Refining Neoantigens for TCR Therapy in Chronic Lymphocytic Leukaemia

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Abstract Text: Chronic lymphocytic leukaemia (CLL) is the most frequent haematological malignancy in adults in Western countries[1]. Despite progress in treatment, including immunotherapies such as CAR-T cells, their clinical benefit in CLL remains limited, underscoring the urgent need for alternative therapeutic approaches[2]. Tumour-specific antigens (TSAs), particularly neoantigens arising from somatic mutations unique to tumour cells, have emerged as a promising, safe, and precise therapeutic target[3]. Identification of exclusive TSAs in CLL patients supports the development of adoptive T cell therapies based on autologous T cells engineered to express tumour-specific T cell receptors (TCRs)[4]. This study aims to establish a reliable method to identify neoantigens, employing pVACbind as a comprehensive computational pipeline that integrates several algorithms for neoantigen prediction[5]. Following in silico prioritization, selected neoantigens will be experimentally validated *in vitro* to identify tumour-reactive TCR sequences. Tumour mutations from 22 CLL patients across various disease stages were analysed. Missense mutations, representing 90.7% of the total, were the primary focus. A strong correlation was observed between the number of unique missense mutations and the predicted number of HLA class I and II neoantigens, suggesting that patients with higher mutational burdens exhibit an increased repertoire of potential neoantigens. To enhance neoantigen prioritization, several filtering steps were applied. RNA-seq data were used to exclude mutations or genes lacking expression. After normalization using DESeq2, a considerable fraction of candidates (26.7% of unique HLA class I and 33.8% of unique HLA class II neoantigens) were discarded. The combined use of pVACbind and NetMHCpan improved stringency compared to previous approaches. Furthermore, 7.8% of prioritized neoantigens were derived from CLL-associated or driver genes annotated in databases. Lastly, information regarding proteasomal cleavage and neoantigen–HLA complex stability for HLA class I neoantigens enabled further prioritization based on mutation location within the peptide. In summary, we present a streamlined pipeline to identify biologically relevant neoantigens in CLL. Our approach enriches for expressed, mutation-driven, and HLA-compatible targets, supporting the development of personalized TCR-based immunotherapies for CLL.

Th158. Intrabodies: A Novel Strategy to Modulate the Expression of PD-1 on CAR-T Cell Therapy

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Abstract Text: CAR T Cell therapy has revolutionized cancer immunotherapy, particularly for hematological malignancies. In this context, the Spanish Agency of Medicines and Sanitary Products (AEMPS) has approved, under hospital exemption, two CAR T Cell therapies developed by Hospital Clínic de Barcelona (HCB): ARI-0001 targeting CD19 and ARI-0002h targeting BCMA. Despite these advances, several challenges still limit the broader application of CAR T Cell therapies in solid tumors. A major obstacle is the immunosuppressive tumor microenvironment, driven in part by inhibitory immune checkpoint molecules (ICPs) such as programmed cell death protein 1 (PD-1). Engagement of PD-1 with its ligands expressed by tumor and tumor-infiltrating immune cells suppresses T Cell function and contributes to CAR T Cell exhaustion. Although gene-editing approaches such as CRISPR have been used to eliminate ICP expression, they raise safety concerns including genotoxicity and off-

target effects. To overcome these limitations, our project explores an alternative strategy based on the intracellular retention of PD-1 to mimic gene knockdown without genomic modification. This approach relies on the generation of chimeric molecules that combine a viral immunoevasin domain, capable of retaining proteins in the endoplasmic reticulum through interaction with TAP, with a PD-1–binding domain. The resulting intrabody prevents PD-1 surface expression, thereby enhancing T Cell functionality. Chimeric constructs were designed and cloned into a third-generation lentiviral vector backbone. Peripheral blood T cells transduced with these vectors were analyzed for PD-1 surface expression and compared to control cells. Several constructs effectively downregulated PD-1, with the best-performing candidate showing consistent and significant PD-1 reduction across multiple healthy donors. This effect was most pronounced between days 3 and 6 post-stimulation, coinciding with peak PD-1 expression in control T cells. Functional characterization included cytotoxicity assays against the acute lymphoblastic leukemia cell line Nalm-6 to assess killing capacity and resistance to exhaustion. Additionally, real-time cytotoxic assays were performed against solid tumor cell lines, including A-549 (lung cancer) and MCF-7 (breast cancer), in the presence or absence of PD-L1. Overall, this study highlights the relevance of the PD-1/PD-L1 axis in CAR T Cell efficacy and proposes intrabody-mediated intracellular retention as a feasible alternative to gene editing for enhancing T Cell–based adoptive cell therapies.

Th159. Profiling the Epigenetic Landscape of Engineered Tregs to Assess Functional Differences Driven by Activation Through Low- vs High-avidity TCR

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Abstract Text: Adoptive cellular therapies, specifically Treg-based cell therapies, offer promise for preventing and treating type 1 diabetes (T1D). Our prior work on engineered Tregs (EngTreg) showed that enforced expression of FOXP3 in conventional CD4 T cells confers regulatory function (Honaker et al, 2020) which can be directed toward islet antigens through introduction of islet-specific TCR (Yang et al, 2022). Notably, we find that functional avidity of the TCR influences activity. Specifically, high-avidity EngTreg have poorer suppressive function than those expressing low-avidity TCR and are characterized in part by the production of pro-inflammatory cytokines upon antigen-specific activation. This is not observed with natural Tregs (nTreg). To understand how enforced FOXP3 expression within conventional CD4 T cells promotes EngTreg regulatory function and how activation through a high-avidity TCR alters that function we profiled the transcriptional and epigenetic landscape of high- and low-avidity islet-specific EngTregs and nTregs following antigen-specific activation. Our paired RNA-seq/ATAC-seq dataset reveals: 1) substantial chromatin accessibility differences between nTregs and EngTregs upon antigen-specific activation, characterized by enrichment of ETS and AP-1 motifs within differentially accessible chromatin in nTreg in comparison to EngTreg , 2) a more modest degree of chromatin accessibility differences between low- and high-avidity EngTregs, but where differential transcription factor occupancy of AP-1, NFκB, and NFAT motifs between low- and high-avidity EngTregs was present. These findings demonstrate that nTregs and EngTregs differentially utilize ETS and AP-1 transcription factors to drive their respective activation programs and suggest that the poorer *in vitro* suppressive capacity found in high-avidity EngTregs stem from these differences. These data also indicate enforced FOXP3 expression alone is insufficient to remodel the epigenome within conventional T cells to match that of nTregs. Further studies are currently underway to identify factors that enable FOXP3 to establish the nTreg epigenetic landscape within EngTregs.

Th160. Plasma Cell Targeting in Autoimmune Disease via CD19xCD3 Bispecific T Cell Engagers: Rapid Systemic Depletion of Autoreactive Antibody Secreting Cells

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Abstract Text: CD19xCD3 bispecific T-cell engagers (BiTEs) represent a transformative strategy for the rapid depletion of B-lineage cells. However, because plasma cells downregulate CD19, the impact of these agents on B cells versus antibody-secreting cells (ASCs) in autoimmunity remains poorly defined. We developed a fully murine CD19xCD3 engager (mCD19xmCD3) that induces complete ablation of $CD19^+$ B cells in healthy and lupus-prone mice, providing an efficient systemic reset. Theoretically, the loss of autoantibodies could be driven by homeostatic turnover of $CD19^-$negative plasma cells following B-cell ablation or by direct targeting of pathogenic $CD19^+$ ASCs. Following a single treatment course, we observed a rapid reduction in autoreactive anti-dsDNA and snRNP serum titers within seven days. To investigate this mechanism, lupus prone mice were evaluated following short-term BiTE treatment. Consistent with direct ablation of CD19-expressing plasma cells, we observed a significant and rapid reduction of splenic ASCs that exceeded 80%. Despite this rapid splenic clearance, subsequent analysis revealed distinct spatial and phenotypic resistance within the bone marrow (BM). Utilizing flow cytometry, we identified a subset of $CD19^-$ $SCA\text{-}1^+$ $IRF4^+$ $CD138^+$ ASCs that remained within the protective BM niche despite BiTE treatment, limiting the extent of absolute autoantibody reduction and serving as a persistent source of pathogenic secretion. Our data suggests that mCD19xmCD3 BiTEs are highly efficient at depleting both $CD19^+$ B cells and $CD19^+$ ASCs, mirroring the rapid clinical responses seen with CD19 CAR T cells. However, an important population of $CD19^-$ negative plasma cells persist in the BM and continues to produce pathogenic antibodies. This study reinforces the potency of BiTEs for B-cell ablation while identifying the BM as a $CD19^-$negative reservoir that may contribute to ongoing disease, informing future strategies for clinical assessment and re-treatment.

Th161. Discovery of HLA Class II–Restricted NY-ESO-1–Specific TCRs Using Single-Cell Transcriptomics and Functional Analyses

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Abstract Text: NY-ESO-1 is an attractive target for T Cell receptor (TCR)-based immunotherapy due to its restricted expression in healthy tissues and broad presence in multiple tumor types. While TCR therapies offer advantages over CAR-T cells by recognizing both intracellular and extracellular antigens, most approaches target HLA class I, which is frequently downregulated in tumors. $CD4^+$ T cells are emerging as critical mediators against solid tumors, yet $CD4^+$ TCR therapies remain underdeveloped. We isolated and functionally characterized antigen-specific TCRs targeting NY-ESO-1119–143 presented on HLA-DRB3*02:02, an allele present in approximately 50% of the Caucasian population. Due to limited access to patient-derived memory T cells, we developed and optimized a robust protocol to stimulate naïve T cells from healthy donors, enabling controlled and reproducible expansion of antigen-specific $CD4^+$ T Cell responses *in vitro*. Naïve HLA-DRB3*02:02^+ $CD4^+$ $CD25^-$ T cells were co-cultured with autologous monocytes pulsed with NY-ESO-1119–143 peptide for 15 days. Antigen-specific T cells were identified by CD4 and CD154 co-expression, sorted, and expanded for an additional 15 days with purity and specificity monitored throughout. Single-cell RNA sequencing (scRNAseq) and TCR sequencing (scTCRseq) characterized the T Cell repertoire and identified TCR candidates. A total of 423 complete TCR sequences were obtained with varying degrees of clonal expansion. To distinguish true antigen-specific TCRs from bystanders, we analyzed phenotypes of the most expanded clones and integrated scRNAseq and scTCRseq data to identify transcriptional features

associated with antigen reactivity. Based on this integrated strategy, 32 TCRs were selected for functional validation. TCRs were cloned into JurkatASP90 reporter cells (NFAT-activated promoter+zsGreen reporter gene) and evaluated through co-culture with K562 cells expressing HLA-DRB3*02:02 and pulsed with peptide. Functional avidity was assessed by titration with varying peptide concentrations. Three TCRs demonstrated peptide-restricted, HLA-specific activation. TCR16 exhibited the highest functional avidity and showed partial CD4 independence, while TCR3 was entirely CD4-dependent and TCR11 displayed intermediate dependence. TCR16 was efficiently transduced into primary T cells via CRISPR-mediated TCR knockout and demonstrated functionality in cytotoxicity assays. This integrated platform successfully identified potent HLA class II-restricted TCRs and validates TCR16 as a promising candidate for clinical development.

Th162. Engineering Immunosuppression-resistant Allogeneic CD19 CAR T Cells to Treat B Cell-driven Autoimmune Disorders Without the Need for Glucocorticoid Weaning

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Abstract Text: Emerging clinical evidence demonstrates that autologous CD19-directed chimeric antigen receptor (CAR) T cell therapy can induce long-lasting remission in systemic lupus erythematosus and other autoimmune diseases by eliminating pathogenic B cells. However, autologous CAR T cell manufacturing is time-consuming, costly, and poorly scalable. Patients with treatment-refractory autoimmune disorders typically receive glucocorticoid-based immunosuppression that require washout prior to T cell harvesting and CAR T cell infusion, leading to treatment delays and increased risk of disease flares. To overcome these limitations, we developed a non-viral, allogeneic CD19-CAR T cell product optimized for co-administration with immunosuppressive drugs. Using our BEKI (Base Editor–mediated Knock-In) platform, we enable simultaneous targeted CAR integration and multiplex gene knockouts with a single enzyme. Confining DNA double-strand breaks to the knock-in site minimizes the risk of deleterious chromosomal translocations and improves the safety compared to conventional nuclease-mediated approaches. We optimized a BEKI strategy to precisely integrate a CD19-specific CAR into endogenous T cell receptor (TCR)/CD3 genes of healthy human donors, while simultaneously modulating their HLA expression to mitigate host immune recognition, and engineering of resistance to glucocorticoids. Combined knockout of B2M and CIITA effectively abrogated HLA class I and II expression, preventing recognition by allo-reactive T cells, but rendered cells susceptible to “missing-self” recognition by NK cells. To address this, we developed base editing strategies for the targeted disruption of HLA-B and HLA¬¬-C while retaining HLA-A on the surface. This strategy effectively reduced NK cell–mediated rejection *in vitro*. Simultaneous glucocorticoid receptor knockout permits immediate administration in patients receiving glucocorticoids. Beyond preserving CAR T cell function, we showed that glucocorticoids suppress allo-reactive T cell and NK cell expansion, further limiting host-mediated rejection of HLA-reduced CAR T cells. *In vitro* co-culture assays revealed reduced monocyte and endothelial cell activation in the presence of glucocorticoids, indicating potential to attenuate the development of cytokine release syndrome (CRS) after allogeneic CAR T cell application. Collectively, this strategy enables a scalable, cost-effective, allogeneic CAR T cell therapy that is ready for immediate use without the need of weaning of glucocorticoid immunosuppression, establishing a promising new treatment paradigm for patients with refractory autoimmune diseases.

Tu146. Dual Targeting of B Cells and Plasma Cells: A CD3xCD20xBCMA Trispecific Antibody for Severe SLE and Autoantibody-driven Diseases

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Abstract Text: Systemic lupus erythematosus (SLE) remains a highly heterogeneous disease with profound unmet needs. Although Mosunetuzumab (CD20/CD3 TCE of Roche), and Teclistamab (BCMA/CD3 TCE of JNJ) demonstrate preliminary efficacy in SLE treatment, each agent induces transient or incomplete responses due to target-specific limitations: Mosunetuzumab depletes $CD20^+$ B cells yet spares immune-privileged long-lived plasma cells (LLPCs) due to their CD20-negative status, whereas Teclistamab eradicates LLPCs but permits B-cell precursor persistence—ultimately driving autoantibody rebound. Theoretically, dual-targeting elimination of B cells and LLPC reservoirs enables durable, treatment-free remission in refractory SLE by interrupting the self-sustaining autoimmune cycle and potentially achieving immune reset. VTS118 is a first-in-class trispecific antibody (TriAb) featuring a proprietary CD3-binding arm that co-targets CD20 and BCMA. *In vitro*, VTS118 depleted autologous B cells more effectively than benchmarks (Mosunetuzumab and Teclistamab) from PBMCs of both healthy human donors and SLE patients. In a PBMC/plasma cell surrogates co-culture assay, VTS118 concurrently depleted B cells and plasma cell surrogates with synergistic activity while benchmarks did not. *In vivo*, VTS118 robustly depleted B cells and plasma cells in pristane-induced lupus mouse model and SLE-patient PBMC-engrafted mouse model, even at low doses. In the dose-range finding (DRF) toxicology study, VTS118 achieved complete depletion of B cells and plasma cells in peripheral blood and lymphoid tissues (including spleen and lymph nodes), accompanied by transient, manageable cytokine release. By simultaneously targeting CD20 and BCMA, VTS118 enables comprehensive depletion of pathogenic B-cell lineages, including precursors and LLPCs, demonstrating promising preclinical potential for severe SLE and other autoantibody-driven diseases. Currently, CMC process optimization for subcutaneous delivery and GLP toxicology studies are advancing. These data support clinical translation, with global IND submission targeted for late 2026.

Tu147. An Integrated Antibody Discovery and LNP-mRNA Platform for Targeted Gene Delivery

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Abstract Text: Efficient and precise delivery is critical for advancing mRNA therapeutics beyond their current applications as vaccines. Here, we aim to enable targeted mRNA delivery by integrating human antibody discovery with high-quality lipid nanoparticle (LNP)-mRNA delivery platforms. To enable precise, targeted mRNA delivery, we developed a highly efficient LNP-mRNA immunization strategy for discovering antibodies against novel and otherwise challenging targets. In parallel, we established a site-specific conjugation technology that enables oriented attachment of antibodies to the LNP surface, including IgG, Fab, scFv, and VHH nanobody formats. To further enhance therapeutic suitability, antibody sequences were optimized through engineering strategies such as humanization and Fc silencing to reduce intrinsic immunogenicity. This immunization strategy enables efficient generation of high-quality antibodies against complex multi-subunit targets, representing a significant improvement over conventional single-subunit protein immunization approaches. We demonstrate the robustness of this strategy across multiple animal models, including humanized mice and camelids. As a proof of concept, we show that antibodies generated through this approach can be successfully conjugated to LNPs, enabling targeted mRNA delivery to $CD5^+$ cells. Compared with conventional random chemical conjugation, our site-specific antibody conjugation method significantly improves the transfection efficiency of LNP-mRNA in target cells. Additionally, antibody sequence optimization, including humanization and Fc silencing, improves overall safety and therapeutic efficacy of LNP-encapsulated mRNAs. Conjugating antibodies to LNPs enables precise and efficient mRNA delivery to desired cell populations. Together, our integrated site-specific LNP conjugation and human antibody discovery platform provides a powerful end-to-end workflow for developing highly targeted and potent mRNA therapeutics.

Tu148. EPI-STOP: Platform for High Throughput Discovery of Post-translationally Modified T Cell Antigens

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Abstract Text: Objective: Protein post-translational modifications (PTMs) can generate clinically relevant neo-antigens in autoimmune diseases and cancer, yet systematic characterization of the PTM antigenic landscape remains limited by the lack of scalable, high-throughput approaches. Here, we sought to develop a platform capable of directly interrogating how PTMs influence peptide immunogenicity, including their effects on major histocompatibility complex (MHC) binding and T cell receptor (TCR) recognition. Methods: We developed EPI-STOP, a cell-based platform for PTM antigen discovery that leverages genetic code expansion (GCE). This approach reassigns the amber stop codon using orthogonal tRNA and aminoacyl-tRNA synthetase (aaRS) pairs to enable site-specific incorporation of non-canonical amino acids (ncAAs) into the epitope sequence of peptide-MHC (pMHC) constructs. We validated the system by incorporating ncAAs, including acetyl-lysine and photocaged citrulline, into defined epitopes. Using EPI-STOP, we generated and screened large libraries of ncAA-containing epitopes in the context of H-2Kb and HLA-A2. To assess disease relevance, TCRs cloned from patients with Rheumatoid Arthritis were evaluated for their ability to recognize citrullinated epitopes presented by disease-associated HLA-DRB1 alleles. Finally, we tested whether the platform could support *in vitro* expansion of PTM-reactive T cells. Results: EPI-STOP enabled efficient, site-specific incorporation of ncAAs into pMHC constructs and provided direct functional readouts of positional PTM effects on both MHC binding and TCR recognition. High-throughput screening identified candidate acetylated neo-epitopes, including peptides predicted to bind poorly in their unmodified form, highlighting the ability of PTMs to reshape antigen presentation. Validation studies demonstrated that patient-derived TCRs specifically recognized their cognate citrullinated epitopes when presented by relevant HLA-DRB1 alleles, even within libraries. In addition, EPI-STOP facilitated the expansion of PTM-reactive T cells *in vitro*, demonstrating its utility for functional immunological studies. Conclusion: EPI-STOP provides a versatile and scalable platform for systematically defining how PTMs shape antigen immunogenicity. By enabling high-throughput, site-specific incorporation of PTMs into epitopes, this approach advances the discovery of disease-relevant antigens and offers broad applications in autoimmune disease and cancer immunology.

Tu149. The Clinical and Islet Reactive T Cell Responses to Anti-integrin Therapy in T1D Are Influenced by Pre-treatment with Anti-TNF Therapy

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Abstract Text: Abstract: Type 1 diabetes (T1D) involves T cell-driven pancreatic beta cell destruction. While immunotherapies can delay onset, questions remain about timing, combinations, and mechanisms. Vedolizumab, an α4β7 integrin–blocking antibody approved for the treatment of inflammatory bowel disease, restricts immune cell trafficking from blood to gut tissue and may alter the distribution of pathogenic T cells in T1D. Objective: To determine if vedolizumab could retain pathogenic islet-specific T cells in peripheral blood in T1D, and whether etanercept (anti-TNF) pre-treatment enhances this effect. Methods: Adults with T1D were randomized to receive vedolizumab alone for 6 weeks (n=11) or etanercept for 8 weeks followed by a 2 week interval and then 6 weeks of vedolizumab (n=9). Clinical response was assessed using quantitative response (QR), which tests whether an individual's insulin secretion is greater or less than expected when controlling for age and baseline insulin secretion, in comparison to hundreds of historical T1D trial placebo participants. Peripheral blood islet reactive $CD4^+$ T cells were quantified using an activation induced marker assay. Results: In the vedolizumab alone arm, islet-reactive memory $CD4^+CD154^+CD137^+CD45RA\text{-}CCR7^{+/-}$ T cell frequencies increased in 7/10 subjects after 10 weeks of vedolizumab (p=0.078). However, in the etanercept pre-treatment arm, islet-reactive T cells declined significantly

after etanercept (p=0.014), returning to baseline before vedolizumab and did not increase after vedolizumab (p=0.87). Etanercept also significantly decreased intermediate monocyte frequency (p=0.014) and HLA expression (p=0.003). Together, these findings suggest that impact of vedolizumab may be different if given immediately after TNF blockade. Consistent with this, QR showed significant clinical effect of vedolizumab across both arms (p=0.019), with a higher trend favoring the vedolizumab only group. Conclusions: This short-term interventional study supports further testing of vedolizumab as a therapeutic candidate for T1D. It also demonstrates that sequential therapies targeting different immune pathways may result in unexpected outcomes, highlighting a need for careful evaluation of combined immunotherapies.

Tu150. Liposome Loaded Dissolvable Microneedle Patch Delivery of a Recombinant Protein Vaccine Against Lymphatic Filariasis

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Abstract Text: Lymphatic filariasis, a tropical parasitic infection causes severe lymphatic pathology and disability. There is no licensed vaccine available for this infection. Our laboratory developed a recombinant protein vaccine (LFGuard), that has now completed cGMP manufacturing. This vaccine is needle-based vaccine and requires cold-chain dependency. Therefore, in this project, we engineered a liposome-loaded vaccine into a dissolvable microneedle (Lipo-dMN) platform for intradermal, and thermostable delivery. Cationic liposomes encapsulating LFGuard were prepared via thin-film hydration, freeze–thaw cycling, and membrane extrusion. The optimized formulation displayed a mean diameter of 264 nm, PDI 0.419, and +3.22 mV zeta potential, with an encapsulation efficiency of ~98%. Antigenic epitope and structural integrity was preserved as confirmed by immunoblot analysis. The hyaluronic acid-trehalose microneedle system (with 1,000 μm pyramidal array) was loaded with the liposomal cargo in the needle tips. SEM analysis confirmed uniform needle morphology. The needles maintained the mechanical robustness suitable for skin penetration. Immunogenicity of the dissolvable microneedle LFGuard vaccine patch was determined in a Balb/c mouse model. Each mouse received three doses of the 15-20 ug of LFGuard protein per patch at two weeks interval, and the titer of antigen-specific IgG antibodies were comparable to the subcutaneous vaccination with the LFGuard vaccine. These findings suggest that the Lipo-dMN LFGuard vaccine is a promising alternative to conventional vaccination, with major advantages including stability of the vaccine antigen, easy transport and is minimally invasive.

Tu151. Decoding Single-cell Protein Interactomes of CAR T Cells with the Proximity Network Assay

Michael Förster and William Love

Pixelgen Technologies

Abstract Text: Proteins on the cell surface are key determinants of the cellular function in the adaptive immune system. The Proximity Network Assay (PNA) is a sequencing-based platform that resolves protein organization at single-cell resolution. Using DNA-barcoded antibodies and proximity-dependent ligation, PNA simultaneously measures the abundance, clustering, and colocalization of 155 immune proteins, generating nanoscale surface maps comprising ~50,000 molecular positions per cell without the use of optics. This spatially resolved readout enables systematic analysis of the membrane proteome across thousands of cells. We demonstrate the utility of PNA by identifying the proxiome of the CD19 CAR receptor at steady state and revealing dynamic proteomic remodeling during tumor cell encounter, including key phenomena such as trogocytosis and cell–cell conjugate formation that are key for CAR T cell function *in vivo*. By integrating spatial context with multiplex protein profiling at scale, PNA provides a powerful platform for protein interactomics, biomarker discovery, and mechanistic insights for cellular immunotherapies currently under clinical evaluation.

Tu152. A Next Generation Immune Cell Engineering Platform to Create Multifunctional Cells at Clinical Scale Using a Silicon Membrane-Based Delivery System

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Abstract Text: Current delivery methods for immune cell engineering, such as viral vectors or electroporation, face limitations in scalability, toxicity, and cargo versatility. To address this, we developed a novel intracellular delivery platform utilizing mechanoporation via silicon membranes. This method causes minimal perturbation to normal gene expression, is compatible across cell types, and has been used to introduce CRISPR RNPs, mRNA, circRNA, siRNA and peptides to primary immune cells and stem cells, including naive and activated T cells, B cells, NK cells, monocytes, iPSC and HSC. To demonstrate multiplexed engineering, we simultaneously delivered mRNA and CRISPR-RNPs to naive T cells, achieving over 85% B2M deletion and GFP expression in live cells. We also show that this method of delivery can enable delivery to cells in whole blood without prior separation, significantly simplifying manufacturing. Using this approach, we achieved over 50% efficiency in generating multifunctional CD19 CAR and membrane-bound IL-2 double-positive T cells when boosted in whole blood, while maintaining over 90% viability. Additionally, we demonstrated simultaneous expression of functional CD19 CAR and membrane-bound IL-2 through the delivery of two circular RNAs. At clinical scale, the platform has demonstrated delivery to more than 1 billion T cells per minute. Given its scalability and compatibility across diverse cell types and cargoes, this approach can reduce manufacturing time and enable unique cell engineering enhancements for next-generation therapeutics.

Tu153. PD-1$^{-/-}$ Hematopoietic Stem/Progenitor Cell Transfer Enhances Efficacy of Whole Brain Irradiation and αPD-1 in a Murine Glioma Model

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Abstract Text: Introduction Glioblastoma (GBM) is among the most lethal primary brain tumors, with consistently poor survival outcomes. Although immune checkpoint inhibitors (ICIs) have transformed the treatment landscape for many solid tumors, their efficacy in GBM has been largely underwhelming. To address this resistance, we developed a combinatorial immunotherapeutic strategy that integrates CRISPR-engineered PD-1-deficient hematopoietic stem and progenitor cells (HSPCs), whole-brain radiation therapy (WBRT), and αPD-1 checkpoint blockade in a syngeneic murine glioma model that is completely unresponsive to ICIs alone. This multi-modal approach significantly prolonged survival in glioma-bearing mice compared to controls. Together, these findings support the potential of multi-pronged immunotherapies to improve outcomes in glioma and identify PD-1 as a promising therapeutic target. Methods C57BL/6 mice were orthotopically implanted with KR158B gliomas and subsequently treated with a single 9 Gy dose WBRT. Bone marrow-derived lineage- HSPCs were isolated and subjected to CRISPR-mediated PD-1 knockdown via electroporation, then co-administered with immune checkpoint inhibition using αPD-1 at a dose of 10 mg/kg. Results Our data demonstrated a significant survival benefit with the combinatorial therapy of PD-1$^{-/-}$ HSPCs, WBRT, and αPD-1, with 50% of glioma-bearing mice surviving beyond 120 days. Immunophenotyping by flow cytometry revealed a reduction in myeloid-derived suppressor cells (MDSCs) within the tumor environment, along with increased frequencies of functional CD8^{+} effector, effector memory, and central memory T cell populations. In addition, expression of the inhibitory receptor LAG-3 within the CD8^{+} population was reduced in mice receiving the combinatorial therapy compared with tumor-only controls. Notably, analysis of thymic tissue revealed increased PD-1^{+} cells within CD8^{+} T cell populations. In cervical lymph nodes, we observed a reduction in central memory CD4^{+} T cells, accompanied by increased CD162^{+} cells in CD4^{+} populations and a corresponding decrease in CD162^{+} populations in CD8^{+} T cells. Conclusion The use of CRISPR-engineered PD-1$^{-/-}$ HSPCs in combination with WBRT $^{+}$ αPD-1 appears to reprogram the immune system, enabling effective ICI responses in otherwise treatment-resistant gliomas. Further studies are needed to elucidate the underlying

mechanisms within the tumor microenvironment and peripheral lymphoid organs, particularly the observed alterations in $CD4^+$ and $CD8^+$ T cell subset distributions.

Tu154. Modeling Treg–ediated Immune Regulation in Human Lymph Node Organoids

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Abstract Text: Human lymph nodes (LNs) are central sites of immune activation, regulation, and alloreactivity, yet most mechanistic insight in these processes is derived from mouse models that incompletely recapitulate human immunity. To establish a human-cell-based system to study LN immune responses we obtained mononuclear cells from human spleen and LNs and established a collagen-based human LN organoid platform to recreate key structural and functional LN features. We found that human LN-derived organoids formed consistently within collagen matrices and maintained comparable T and B cell proportions without selective loss of major immune subsets. To enable post-culture flow cytometric analysis, we tested various collagen digestion methods, revealing that use of collagenase D enabled efficient cell recovery without loss of key lineage or activation markers. Flow cytometric analysis demonstrated comparable T and B cell populations after one week of culture. Confocal microscopy of intact organoids revealed spatially distributed T cells, B cells, and dendritic cells, suggesting the preservation of cellular networks. To assess immune regulation, human regulatory T cells (Tregs) were labeled with cell-tracker dyes and introduced into collagen-embedded organoids. Imaging demonstrated efficient Treg migration and penetration throughout the 3D structure, indicating accessibility of regulatory cells to immune niches within established organoids. Together, these results establish a human LN organoid system that can be used to study and visualize the dynamics of immune regulation. This platform also provides a tractable, human-focused approach to evaluate the function of engineered Treg therapies for a variety of disease contexts.

Tu178. Generation of CRISPR/Cas9-engineered Anti-IL13Rα2 PD-1-disrupted CAR-T Cells

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Abstract Text: Glioblastoma represents the most prevalent primary malignant brain tumour in adults and is characterized by a poor prognosis and limited overall survival. A promising immunotherapeutic strategy is the use of anti-IL13Rα2 CAR-T lymphocytes, an antigen overexpressed in high-grade gliomas. One such approach is the anti-IL13Rα2 CAR ARI-0008 developed at the Hospital Clinic of Barcelona, currently at preclinical stage. However, one major challenge in treating solid tumors is the ability of T Cells to infiltrate and persist within the immunosuppressive tumor microenvironment. Various therapeutic approaches, such as immune checkpoint inhibitors, have been explored. Blockade of Programmed Cell Death protein 1 (PD-1) in T Cells contributes to restoring their activity, helping them to better confront these highly immunosuppressive environments and enhance tumor cell clearance. PD-1 inhibitors have demonstrated improved anti-tumor immune responses in glioblastoma patients, leading to increased survival. CRISPR/Cas9 genome editing technology allows precise transgene insertion and the modification or disruption of key endogenous genes, such PD-1. This project aims to develop anti-IL13Rα2 PD-1-disrupted CAR-T cell therapy using CRISPR/Cas9 technology. Primary T Cells were isolated from buffy coats and stimulated. Two days later, cells were electroporated with CRISPR/Cas9 technology targeting the PDCD1 gene and a DNA donor template encoding the anti-IL13Rα2 ARI-0008 CAR construct. The percentage of PDCD1 knockout was assessed by sequencing analysis. CAR expression was assessed by flow cytometry on days 6 and 9 post-electroporation. The cytotoxic activity of CRISPR/Cas9-generated CAR-T cells was compared to traditional lentiviral-

transduced CAR-T cells against the IL13Rα2$^+$ PD-L1$^+$ U251 cell line at different effector-to-target ratios. Cytotoxicity was measured via bioluminescence at 24 and 48 hours. A high level of PDCD1 disruption was successfully achieved in CRISPR/Cas9-engineered CAR-T cells. CAR expression remained stable between days 6 and 9 in CRISPR/Cas9-generated CAR-T cells. At high effector-to-target ratios, there were no significant differences in cytotoxicity between CRISPR/Cas9 and lentiviral-generated CAR-T cells. Preliminary results suggest that anti-IL13Rα2 CRISPR/Cas9-generated CAR-T cells exhibit similar cytotoxic activity to lentiviral-generated cells, indicating the potential of CRISPR/Cas9 for CAR-T cell engineering in glioblastoma therapy.

W144. Antibody-targeted Lipid Nanoparticles Enable B Cell-selective mRNA Therapeutic Delivery *in vivo*

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Abstract Text: Autoimmune diseases affect over 15 million individuals in the US alone, representing around 8% of the population, and the incidence is steadily rising. In situ therapeutic mRNA delivery to B cells offers a lineage-specific therapeutic opportunity to treat B cell-mediated conditions. In recent years, lipid nanoparticles (LNPs) have emerged as leading FDA-approved nonviral carriers for mRNA delivery, providing a versatile platform. Here, we use machine learning and rational surface engineering to optimize antibody-conjugated LNPs (bLNPs) for B cell-specific transfection *in vivo*. In humanized models, bLNPs achieved ~25% mCherry$^+$ B cells in the liver, approximately 10-fold higher than benchmark LNPs, whereas the bone marrow showed ~8% mCherry$^+$ expression, an 8-fold enhancement, measured by flow cytometry. In the liver and bone marrow, B cells accounted for roughly 70% and 40%, respectively, of all mCherry$^+$ cells. Bispecific T cell engagers (BiTEs) are exceptionally potent immunotherapeutics that can induce T cell-mediated killing of target B cells through simultaneous engagement of T cell receptor complexes (e.g., via CD3) and B cell lineage antigens (e.g., CD19). We hypothesized that delivering CD19xCD3 BiTE-encoding mRNA to B cells in situ would enable localized, transient, and selective B cell depletion. We tested therapeutic activity in humanized mice treated with a single i.v. dose of 0.5 mg/kg mRNA-bLNPs 4 days after engraftment with human T cells and leukemic B cells (NALM6). Bioluminescence imaging revealed that bLNP-treated mice exhibited marked B cell depletion with 70% reduction in signal within 16 h. Flow cytometry confirmed robust therapeutic effect in tissues. In the liver, BiTE-bLNPs mediated >75% cancerous B cell depletion, compared to ~50% with benchmark BiTE-LNP and 0% with mCherry-bLNP controls. In bone marrow, BiTE-bLNPs mediated >99% depletion of cancerous cells, demonstrating potent localized activity. We present an antibody-decorated bLNP that enables robust and selective mRNA delivery to B cells *in vivo* after a single intravenous dose, supporting efficient in situ expression of therapeutic proteins and demonstrating potent, localized immunotherapeutic activity through transient B cell programming. Together, these findings establish antibody-targeted bLNPs as a modular, nonviral strategy for B cell-specific mRNA delivery and programmable immunotherapeutics.

W145. Engineering Stable and Lymphodepletion-resistant Regulatory T Cell Therapy for Inducing Immune Tolerance in Transplantation and Autoimmunity

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Abstract Text: Organ transplantation is the only treatment option for patients with irreversible organ failure. Transplant recipients typically rely on expensive immunosuppressive drugs to prevent rejection, which pose serious risks, including cancer and infections. Despite this, long-term graft survival is limited by chronic rejection. Therefore, new therapeutic strategies that induce immune tolerance more precisely and safely remain a key goal to achieve.

Regulatory T cells (Tregs) play a crucial role in maintaining immune homeostasis by inducing targeted suppression of immune responses. Despite their promising potential, clinical implementation of Treg therapies is posed with two major challenges: First, lymphodepleting conditioning regimens administered to transplant recipients can deplete Tregs in addition to effector T cells (Teff), causing loss of tolerance to the graft. Second, inflammatory microenvironments can destabilize Tregs and convert them into pro-inflammatory Teff, which could be detrimental for the graft. Using CRISPR/Cas9 editing, we deleted CD2 (a co-stimulatory receptor) from Tregs to render them resistant to siplizumab-mediated lymphodepletion. We found that CD2-KO Tregs retained suppressive functions comparable to WT Tregs, both *in vitro* and *in vivo*. However, they exhibited enhanced oxidative phosphorylation but decreased glycolysis, indicating a stable and 'persistent' type of metabolic profile. Additionally, CD2-KO Tregs show improved mitochondrial fitness with lower production of reactive oxygen species, as well as decreased expression of pro-inflammatory and senescence-associated genes compared to WT Tregs (as indicated by RNAseq). Upon chimeric antigen receptor (CAR)-induced signaling, CD2-KO Tregs retained higher expression of Treg lineage and stability markers (e.g., FOXP3 and HELIOS) but lower expression of exhaustion markers (e.g., PD-1 and γH2AX) compared to WT Tregs. Additionally, CD2-KO Tregs showed lower levels of pro-inflammatory cytokines and decreased lineage conversion when cultured under conditions promoting Th1/Th17 differentiation. We observed that that the phenotype, functionality and metabolism of CD2-KO Tregs exhibited a trend resembling rapamycin-treated Tregs, implicating a mechanism wherein the PI3K-AKT-mTOR axis is potentially downmodulated. Phosphoproteomic analysis revealed decreased mTOR-mediated signaling as an underlying mechanism for the improved metabolic phenotype and lineage stability of CD2-KO Tregs. Together, these findings have potential to advance clinical implementation of Treg-based therapies in organ transplantation as well as autoimmune disorders.

W146. Dual Targeting of Lymphocyte Gut-homing and T Cell Activation: VBS123, a Novel α4β7 X TL1A Bispecific Antibody for IBD

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Abstract Text: To address the efficacy ceiling in Inflammatory Bowel Disease (IBD) therapy - particularly in patients with activated fibroblast signatures who are non-responsive to vedolizumab (anti-α4β7; approved in 2014) - we developed VBS123, a bispecific antibody targeting both α4β7 and TL1A. VBS123 is designed to synergistically block lymphocyte gut-homing while inhibiting pathogenic T Cell activation and intestinal fibrosis, thereby remodeling the inflammatory microenvironment more effectively than monotherapy or combination approaches. The molecule was engineered using proprietary sequences and technology. VBS123 molecule acted as a potent dual blocker. In an IFN-γ release assay using human PBMCs stimulated with TL1A and other cytokines to model gut inflammation, it synergistically suppressed $CD4^+$ T Cell activation, outperforming both parental controls and their combination. In cell-based assay, it promoted the α4β7- mediated internalization and degradation of both soluble TL1A and the α4β7 receptor itself, generating a 'sink effect' to further clear circulating cytokines. *In vivo*, VBS123 demonstrated superior therapeutic efficacy over the benchmark mAb combination of vedolizumab and afimkibart (anti-TL1A; Phase 3) in both TNBS-induced (Crohn's Disease-like) and DSS-induced (Ulcerative Colitis-like) acute murine colitis models. Across both models, VBS123 treatment significantly ameliorated the Disease Activity Index (DAI), mitigated body weight loss, and improved colon index; these clinical benefits were further corroborated by superior histological outcomes. By concurrently preventing immune infiltration and suppressing local T Cell activation, VBS123 delivers coordinated microenvironment remodeling and shows best-in-class potential in preclinical IBD models. Currently, GLP-compliant toxicology studies and CMC development for subcutaneous administration are underway. These data support its further clinical development, with a U.S. IND submission planned for late 2026.

W147. Unveiling Off-target Co-stimulation-dependent Activities of CD19-CAR T Cells

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Abstract Text: Introduction CD19 chimeric antigen receptor (CAR) T cell therapy represents a paradigm shift in the treatment of oncological and autoimmune diseases. Despite its clinical success, CAR T cell therapy can be associated with significant toxicities highlighting the need to better understand their mechanism and find strategies to mitigate them. Herein, we evaluate the impact of interactions between CD28 and B7 family molecules (CD80 and CD86) on CD19-independent CAR-T cell activities, and the role of the CYS-T motif present in the CD28 transmembrane domain (TMD) in mediating the formation of CAR-CD28 heterodimers. Methods We generated second-generation lentiviral constructs encoding anti-CD19 CARs with selective mutations in the CYS-T motif (M4 construct), using either a 4-1BBζ or CD28ζ intracellular domain (ICD). Various CD19-deficient or CD19-negative target cell lines were engineered to express CD80, CD86, or both. We then performed activation, cytotoxicity, *in vitro* proliferation, and *in vivo* migration assays. Results Expression of the wild-type (WT) CAR, but not the M4-based CAR, was associated with a significant decrease in the detection of cell surface CD28, suggesting heterodimerization with the CAR. CD80, but not CD86, triggers the activation of the NFAT, NF-κB and AP-1 pathways in WT-TMD CD19 CAR, independently of the ICD. In primary T cells, contact with CD80 expressing cells induced significant CD25 and CD71 upregulation, T cell proliferation, and cytotoxic activity, independently of CAR binding to CD19. These results are consistent with the capacities of CD19 CAR T cells to uptake CD80 molecules through a trogocytosis-like mechanism. Importantly, CD28 knockout abrogated B7-induced off-target activation. Strikingly, WT-TMD CAR T cells, but not M4 variants trafficked to CD80-expressing tumors *in vivo*. Finally, co-administration of CTLA4-Ig prevented off-target activation, proliferation and *in vivo* migration. Conclusions Collectively, these findings indicate that WT-TMD-containing CARs are susceptible to antigen-independent activation through CD80 interactions. Further experiments are ongoing to evaluate the relevance of these finding in the context of primary dendritic cells and stem cell-derived microglial cells. The modulation of TMD structure and the use of CTLA4-Ig represent promising strategies to enhance CAR-T cell specificity and safety, particularly in the context of autoimmune diseases.

W148. Automated Antibody Cocktailing and C-FREE™ Workflow for Immuno-engineering Applications

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Abstract Text: Engineered immune-cell therapies such as CAR-T and NK-based products rely on high-parameter flow cytometry to assess phenotype, activation state, and product quality. However, antibody cocktailing and sample-preparation workflows remain highly manual and difficult to standardize, contributing to variability in immunophenotyping results across operators and study days. Automation of these preparative steps offers a practical path toward more consistent execution of complex immuno-engineering assays. The Pluto workstation provides a UI-driven, SOP-based framework that automates antibody cocktailing and integrates seamlessly with C-FREE™, an end-to-end sample-preparation workflow that standardizes staining, mixing, and washing steps. Using spreadsheet-like configuration tables, users define reagent mapping, mixing logic, and volume scaling without requiring any scripting or coding expertise. This lowers the barrier for implementing multi-marker workflows and enables reproducible preparation of cocktails relevant to engineered T- and NK-cell characterization. Automated workflows also support large-volume cocktail generation and record all executed actions through an integrated audit trail to facilitate traceability and version control. We characterized the operational reproducibility of automated cocktail preparation and C-FREE sample handling through replicate preparations, stability evaluations, and workflow scalability assessments. Across 10- to 24-marker panels, automated cocktailing eliminated all hands-on cocktail preparation while maintaining SI performance with CV% comparable to manual preparations, minimizing operator-driven variability and reducing opportunities for mixing errors. Collectively, these data indicate that a unified

automation-based workflow can support consistent immunophenotyping of engineered immune cells while lowering manual burden and providing a scalable foundation for harmonized execution in research and development environments. This work highlights the role of workflow automation in enabling reliable, high-parameter flow cytometry for immuno-engineering applications without relying on multi-week manual panel preparation cycles.

W149. Validation of High Throughput Microneutralization Assay for anti-AAV2 Neutralizing Antibodies (NAbs) in Clinical Trials in Support of Gene Therapy

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Abstract Text: Background Humoral immunity against the adeno-associated (AAV) develops throughout life and levels varies by age, geographic location, race and serotype. AAV vectors have become common for delivering therapeutic articles, e.g. in gene therapy. The presence of pre-existing NAbs inhibits the binding of therapeutic viral vectors to the cells resulting in the failure of therapeutic gene delivery. Titers of pre-existing anti-AAV antibodies (NAbs) can be exclusion criterion in AAV-based treatments. One important factor that may influence the sensitivity of the assay readout is the type of the *in vitro* measurement method used to quantify the NAbs. Objective Commonly used NAbs assay is a luciferase-based assay. The latter measures the transfected cells lysed in a liquid solution, which is then evaluated by an additional substrate using an ELISA readout system. Hence this system is not measuring the individual transfected cells directly. In our anti-AAV2 NAb assay the GFP expressing cells are counted directly, without any additional steps. Design/Methods The Green Fluorescent Protein (GFP)-carrying AAV2 vector (Reporter Virus Particles- RVP) was used. The vector was incubated with the positive/negative sample controls and with the serially diluted test sample. These mixtures were added to the target cells for an additional incubation period and GFP expressing cells were counted thereafter. A sample was considered positive for NAbs with a reduction of vector transduction greater or equal to 50%. Results We tested 78 samples using different sample types CSF, Serum, Plasma. Established positive, negative samples and NAb titers. We showed specificity of the assay and validated the anti-AAV2 neutralizing antibodies (NAbs) using antigen specific positive and negative samples. Conclusions The validated CTL anti-AAV2 NAb assay provides a direct readout for fluorescent cells transduced with AAV2 GFP RVP in a high throughput assay format. This approach is simpler, less variable, offers high throughput and is a highly sensitive assay detecting neutralizing anti-AAV2 antibody. In conclusion, we developed a highly reliable and sensitive anti-AAV2 NAb assay with direct enumeration of fluorescent AAV2-GFP transduced cells, which allows sensitive, high throughput evaluation in preclinical as well as clinical trials.

W150. Functional Characterization of CD19 CAR T Cells for the Treatment of Systemic Lupus Erythematosus

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Abstract Text: Chimeric antigen receptor (CAR) T cells have rapidly become one of the most promising immunotherapeutic approaches for the treatment of relapsed or refractory hematological malignancies such as multiple myeloma, B cell acute lymphoblastic leukemia, and B cell lymphoma. The remarkable success of CD19 and BCMA-specific CAR T cell in oncology has prompted the exploration of their therapeutic potential against autoimmune diseases driven by autoreactive B cell lineages. Systemic lupus erythematosus (SLE) is a chronic B cell-related autoimmune disease characterized by the production of autoantibodies, innate and adaptive immune system dysregulation, and progressive organ damage. Recently, early phase CD19 CAR T cell clinical trials have enabled the treatment of a small number of patients with SLE and have resulted in B cell depletion and serological remission. However, given that SLE can lead to T cell dysregulation, mitochondrial dysfunction, and altered metabolic programming, understanding its effect on CAR T cell therapy is essential for effective treatment. Patients with SLE experience chronic exposure to inflammatory molecules, such as type I interferon and immunomodulatory

drugs, that result in altered T cell metabolism, function, and phenotype compared to healthy donors. To investigate the impact of SLE-associated T cell abnormalities on CAR T cell therapy, we plan to use both human samples and mouse models. We aim to generate CAR T cells from SLE, non-Hodgkin lymphoma, and healthy donors and assess T cell phenotype, functionality, and susceptibility to exhaustion. Moreover, we will also explore the relationship between disease severity and CAR T cell efficacy in murine models of SLE by generating CAR T cells at different points of disease progression, from early inflammatory stages to established disease. Ultimately, this project will guide the development of optimized, next-generation cell-based therapies for the treatment of SLE and other autoimmune diseases.

W151. Single-cell Fate Trajectories of Engineered CD4LVFOXP3 Treg Cells from Clinical-grade Production to Post-treatment in Patients with IPEX Syndrome

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Abstract Text: FOXP3^{+} regulatory T cells (Tregs) are central to immune tolerance, yet the development of effective Treg-based cellular therapies has been limited by an incomplete understanding of how expanded or engineered Tregs are generated and function *in vivo*, particularly in the treated patients. This challenge is compounded by the lack of traceable cell products that enable longitudinal analysis during manufacturing and after infusion. Here, we combined flow cytometry and single-cell multi-omics to track a lentiviral-engineered, traceable CD4^{+} T cell product expressing FOXP3 and a truncated nerve growth factor receptor (tNGFR) marker (CD4LVFOXP3) from manufacture through six months after infusion in eight patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome (NCT NCT05241444). At the end of manufacturing, CD4LVFOXP3 product preserved parental cellular heterogeneity and T cell receptor (TCR) repertoire diversity, while acquiring stable Treg-associated transcriptional programs, anergic features, cytokine restriction, and suppressive capacity *in vitro*. CD4LVFOXP3 products are enriched for a naïve-derived cell population with features of T stem cell memory, driven by ectopic FOXP3 expression rather than culture conditions. Post infusion, CD4LVFOXP3 cells maintained high and stable FOXP3-lv transgene expression independent of TCR signaling. Non-activated Tscm CD4LVFOXP3 cells likely returned toward a naïve state and expressed high levels of lymphoid homing genes, whereas expanded CD4LVFOXP3 cells displayed clonal specificity, stable effector Treg phenotypes and tissue- or inflammation-associated homing programs, suggesting they are capable of migrating to inflamed tissues. Together, these findings define key principles of engineered human Tregs and establish CD4LVFOXP3 cells as a mechanistically informed platform for Treg-based immunotherapy.

W152. *In vitro* T- and B-cell Immunogenicity Profiling of Biologics Using a High Throughput Screening Platform and 3D Secondary Lymphoid Organoid Cultures

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Abstract Text: Many biotherapeutics have the potential to be recognized as foreign by the human immune system, leading to the development of anti-drug antibodies (ADAs). This undesirable immunogenicity can limit clinical success by modifying the drug's pharmacokinetic (PK) profile and, in some cases, cause adverse health effects. ADA generation is typically initiated when a biotherapeutic contains immunogenic epitopes capable of activating CD4 T cells, which in turn stimulate B cells, promoting their differentiation into plasma cells that secrete drug-specific antibodies. To address this challenge, we highlight two *in vitro* platforms designed to assess the immunogenicity risk of biotherapeutics. The first model addresses the initial immunogenicity by priming T cells through culturing primary

human peripheral blood mononuclear cells (PBMCs) with a test compound and quantifying the CD4 T cell response. This system enables comparative benchmarking against reference biotherapeutics with clinically established ADA profiles. Here, we present data using a panel of antibodies with known clinical immunogenicity data against a broad range of donors and HLA-haplotypes. The second approach employs a human tonsil-derived organoid platform to assess immunogenicity risk of biotherapeutics, with a particular emphasis on antibody therapeutics. This system captures key features of human B cell biology, including affinity maturation, somatic hypermutation, and antibody secretion, within a physiologically relevant tissue microenvironment. By preserving diverse B cell repertoires alongside supporting immune cell populations, the platform enables evaluation of biologic-driven humoral responses that complement T cell–centric risk assessment. Similarly, the platform supports benchmarking using therapeutic antibodies associated with varying degrees of clinical ADA incidence, enabling comparative ranking of candidate molecules based on relative immunogenicity risk. When integrated with upstream T cell priming assays, the tonsil organoid platform provides a scalable, non-animal, human-relevant framework to inform candidate selection, molecular design, and early immunogenicity de-risking in biologics development. These human cell-based assays facilitate translationally relevant immunogenicity assessment across a broad range of human HLA haplotypes, aligning with the New Approach Methodologies (NAMs) now favored by regulatory agencies like the FDA. Overall, assessing immunogenicity risk early in drug development can mitigate downstream risks and enhance clinical success.

W153. An Integrated Platform for the Identification of Neoantigen-specific TCRs Enabling Transgenic TCR-T Cell Therapy in Chronic Lymphocytic Leukemia

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Abstract Text: Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults, with a high incidence in elderly populations. The adoptive transfer of autologous T cells genetically modified to express tumor-specific T cell receptors (TCRs) represents a promising personalized immunotherapy. However, its clinical implementation is limited by the time-consuming identification of neoantigens and their corresponding tumor-specific TCRs. This study aims to develop and optimize an integrated platform for the rapid identification of neoantigen-specific TCRs and their generation as transgenic TCRs (tTCRs) for personalized CLL therapy. Tumor mutations from CLL patients were analyzed to predict class I- and class II-restricted neoantigens using pVacbind, a bioinformatic tool that integrates multiple HLA-binding prediction algorithms. TCR repertoires from CLL patients at different timepoints were analyzed and compared with the predicted neoantigen–HLA complexes. Candidate TCRs were selected using an in silico approach that integrates multiple bioinformatic tools to predict TCR specificity and to compare candidate receptors with neoantigen-derived epitopes identified at the corresponding disease time point for each patient. In parallel, an *in vitro* strategy was implemented consisting of a 14-day peptide stimulation protocol optimized using T cells from healthy donors and validated with patient-derived T cells. CD154 and CD137 were used as activation markers for $CD4^+$ and $CD8^+$ T cells, respectively. Finally, we are developing a fully in-house platform to enable the selection, cloning, and expression of candidate TCRs in T cells through lentiviral transduction, using vectors generated with the BioXP system. Through this integrated bioinformatic and experimental pipeline, several candidate neoantigen-specific TCRs were successfully identified, representing promising targets for further functional validation. Overall, this study highlights the value of combining computational prediction tools with experimental validation to streamline TCR candidate selection, a major bottleneck in personalized T cell therapies. The optimized platform described here may enhance the feasibility of personalized immunotherapy for CLL and has potential applicability to other oncological diseases. This project (PMPTA23/00027) is funded by the Instituto de Salud Carlos III within the framework of the Plan de Recuperación, Transformación y Resiliencia, with support from the European Regional Development Fund.

Immunogenetics

Th163. Engineering Heterozygous Variants to Decode Variable Penetrance in Immune Disorders at Single Cell Resolution

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Abstract Text: Introduction: Heterozygous single-nucleotide variants cause many inborn errors of immunity (IEI) and account for a major source of diagnostic uncertainty and variable penetrance, yet most functional genomics tools generate homozygous edits, limiting insight into dominant negative (DN) and dosage-dependent variant effects. Cell-to-cell and patient-to-patient variability in expression of the mutant versus wild-type allele has emerged as a driver of genotype-phenotype discordance and variable disease penetrance but has been nearly impossible to interrogate experimentally. To overcome this, we built a scalable platform to engineer heterozygous variants in primary immune cells and directly link allelic expression bias to phenotype. Methods: We harnessed a scalable CRISPR-based editing platform, helicase-assisted continuous editing (HACE), in primary human T cells to engineer heterozygous variants across CARD11, a key regulator of immune signaling, in which heterozygous variants cause diverse IEI with variable penetrance. In parallel, we developed single-cell Allele-Integrated Multi-omics sequencing (sc-AIMseq), which integrates transcriptomics, surface proteomics, and direct quantification of mutant and wild-type allele expression within the same single cells. Results: HACE screens robustly recovered known pathogenic DN variants in CARD11 and uncovered a broad landscape of novel DN and haploinsufficient variants that were impossible to distinguish in homozygous perturbation models. sc-AIMseq of HACE-edited T cells uncovered marked cell-to-cell variability in CARD11 allelic expression bias, which directly dictated T cell activation state, signaling profile, and phenotype. We also performed sc-AIMseq on PBMCs from multiple patients with DN CARD11 mutations, which revealed that CARD11 allelic expression bias is highly variable and strongly cell-type dependent, providing a direct mechanism to explain the variable penetrance seen in patients with identical CARD11 genotypes. Conclusion: This work establishes a uniquely scalable strategy to model heterozygous genetic disorders in primary and patient-derived immune cells and dissect their variable penetrance. By directly coupling heterozygous variant engineering, quantification of allelic expression bias, and deep phenotyping at single-cell resolution, our approach enables unprecedented functional interpretation of heterozygous variants and lays the foundation for improved diagnosis, risk stratification, and therapeutic decision making in IEI and beyond.

Tu155. Biological Mechanism and Treatment of Spondyloenchondrodysplasia (SPENCD)

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Abstract Text: Spondyloenchondrodysplasia (SPENCD) is a unique inborn error of immunity, causing immune, skeletal, and neurological dysfunction. SPENCD is caused by damaging variants in the gene ACP5, which encodes tartaric-acid-resistant acid phosphatase (TRAP). TRAP has dual roles as a phosphatase and a generator of reactive oxygen species (ROS). We hypothesize that damaging ACP5 variants impair both functions. A new SPENCD case presented at age 2 years with autoimmune hemolytic anemia, splenomegaly, short stature, developmental delay, intracranial calcification, and skeletal dysplasia. Compound heterozygous ACP5 variants were identified on next-generation sequencing (NM_001611.5: c. 550C>T, p.(Q184*) and c. 740T>G, p.(L247R)). Site-directed mutagenesis was used to generate ACP5-variant-expressing constructs for patient and known SPENCD variants. TRAP production (immunoblotting) and phosphatase activity (p-nitrophenyl phosphate assay) were assessed in transfected HEK293 cells. Flow cytometry with ROS staining (H2DCF) was performed on PBMCs and THP-1 cells treated with

TRAP Inhibitor (AubipyOMe). p.(Q184*) produced truncated protein; however, p.(L247R) expression was normal. Phosphatase activity was impaired for all SPENCD variants. ROS production was impaired in AubipyOMe-treated cells. We present a new SPENCD case, demonstrating the dual roles of TRAP, and providing the evidence to reclassify p.(L247R) as damaging. Baricitinib, a JAK inhibitor, may be an effective treatment targeting immune overactivity. Our work-in-progress harnesses multi-omics (RNA sequencing and mass spectrometry), flow cytometry (SIGLEC1 inflammatory biomarker), and type I IFN score to assess baseline and post-baricitinib treatment.

Tu156. Investigating Potential Therapeutic Targets in the Pathway Underlying Pathogenic Association Between PRKG1 Variants and Type I IFN Production in SLE

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Abstract Text: Interferon (IFN) α is a key pathogenic factor in SLE. The PRKG1 rs7897633 variant has been associated with elevated IFNα levels in SLE patients of European ancestry, although the underlying mechanism is unclear. PRKG1 encodes cGMP-dependent protein kinase I (PKGI), an inhibitor of RhoA and Rho-associated kinases (ROCKs). The RhoA/ROCK pathway regulates cytoskeletal reconstruction, which influences cell clustering and T cell development and function. While ROCK inhibitors reduce IFNα production, they also non-selectively suppress T cells. Therefore, we investigated PKGI as a potential therapeutic target in SLE patients carrying the rs7897633 variant. PBMCs from SLE patients and healthy controls were isolated by Ficoll-Paque density centrifugation and pre-treated with a PKGI agonist (PKGIa; 8-pCPT-cGMP), a pan-ROCK inhibitor (ROCKi), Y27632, or a selective ROCK II inhibitor (ROCK2i), KD025, for 1 hour. Cells were then stimulated with media, R848, or ODN 2216 for 16–18 hours. For plasmacytoid dendritic cell (pDC) analysis, Brefeldin-A was added. pDCs were stained for intracellular IFNα and analyzed by flow cytometry. For regulatory T cell (Treg) and T helper (Th)17 analyses, PBMCs were stimulated with plate-bound anti-CD3 or anti-CD3 plus anti-CD28 in the presence of PKGIa, ROCKi, or ROCK2i. On day 7, cells were stained for surface and intracellular markers. Gene expression of PRKG1, ROCK1, ROCK2, and RhoA in healthy controls was assessed by RT-qPCR. Treg expansion in both healthy controls and SLE patients was unaffected by PKGI but significantly reduced by ROCKi and ROCK2i ($p < 0.05$). Th17 cell numbers were also unaffected by PKGI stimulation. In SLE patients, PKGIa did not significantly alter the number of IFNα-producing pDCs compared to untreated cells. In contrast, ROCKi markedly reduced IFNα-producing pDCs, while ROCK2i produced a significant but lesser reduction ($p < 0.05$). Preliminary gene expression data showed a trend toward lower PRKG1 and higher ROCK1, ROCK2, and RhoA expression in homozygous AA compared to CC genotypes, although statistical significance is not reached. Overall, PKGI stimulation does not affect T cell expansion or IFNα-producing pDC numbers, despite an association with higher IFNα signaling. These findings suggest that the PRKG1 variant may influence SLE pathogenesis through alternative mechanisms, such as cytoskeleton-driven clustering of IFNα-producing cells.

Tu157. Adult-onset Severe Hypogammaglobulinemia with Profound B-cell Lymphopenia and Marked CD21^$_{low}$ B-cell Expansion Presenting with Recurrent Infections and Chronic Diarrhea

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Abstract Text: Background: Common variable immunodeficiency (CVID) is the most frequent symptomatic primary antibody deficiency in adults and is characterized by hypogammaglobulinemia, impaired specific antibody responses, and increased risk of non-infectious complications. Expansion of CD21^$_{low}$ B cells has been associated with immune dysregulation phenotypes. Case: A 46-year-old woman was evaluated for recurrent infections and chronic diarrhea. Serum immunoglobulins were profoundly reduced: IgA undetectable (< 0.1 g/L), IgG 3.22 g/L, and IgM < 0.2 g/L (units as reported). Absolute lymphocyte count was 1.2×10^9/L. Flow cytometry demonstrated marked

B-cell lymphopenia with relative T Cell predominance: CD3^+ 89.6% (≈1075/µL), CD4^+ 61.8% (≈741/µL), CD8^+ 26.6% (≈320/µL), CD19^+ 3.36% (≈40/µL), and CD20^+ 3.45% (≈41/µL). B-cell subset analysis revealed low class-switched memory B cells (IgD^−CD27^+ 4.59%) and striking expansion of CD21^$_{low}$CD38^$_{low}$ B cells (46.28%). T Cell maturation profiling showed reduced naïve CD4^+ cells and low recent thymic emigrants (RTE 6.83%). Double-negative T cells (TCRαβ^+CD4^−CD8^−) were borderline increased (2.7%). A next-generation sequencing panel for immune dysregulation/eczema identified no pathogenic/likely pathogenic variants; a heterozygous IRF8 p.Ile15Met variant of uncertain significance was reported. Conclusion: The combination of severe panhypogammaglobulinemia, profound B-cell lymphopenia, reduced switched memory B cells, and marked CD21^$_{low}$ expansion is highly compatible with a CVID-like adult-onset antibody deficiency with potential immune dysregulation. The case underscores the value of extended B-cell phenotyping in adults with recurrent infections and chronic diarrhea and supports early comprehensive evaluation to exclude secondary causes and guide immunoglobulin replacement therapy.

W154. Beyond the Germline: Mapping Somatic Causes of Rare Inflammatory Disease in Adults

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Abstract Text: Background: Inborn errors of immunity (IEIs) are monogenic disorders of immunity. IEIs are generally considered inherited, however, somatic mutations acquired later in life can also lead to IEI-like disorders, or “phenocopies”. Their true prevalence remains unclear. We aimed to estimate the prevalence of phenocopies of IEIs in an adult population. Methods: We analyzed somatic mutations from whole-exome sequences from 59,000 Mount Sinai Million Biobank participants using Mutect2. After validation of pipeline performance, we evaluated genotype–phenotype relationships for 21 IEI genes with known somatic disease associations by linking variant data to electronic health records (EHRs). Results: We identified 139 protein-altering somatic mutations across 21 genes. After manual review of EHRs, 30 variants were classified as putative pathogenic based on the presence of associated phenotypes. Chart review was blinded to previous pathogenicity reports, yet 10 (33%) of these variants were previously reported as pathogenic. These 10 variants have a mean variant allele fraction (VAF) of 15.1% and a high deleteriousness score (mean CADD 26.5). Notably, the remaining 20 pathogenic variants are novel, yet exhibit similar characteristics including a VAF of 15.6% and high predicted deleteriousness (mean CADD 22). Laboratory records corroborated the inflammatory phenotype, showing elevated erythrocyte sedimentation rate in 14 of 20 (70%) and C-reactive protein in 6 of 20 (30%) individuals harboring novel variants. Conclusions: We identified known and novel putative pathogenic somatic mutations underlying phenocopies of IEIs. Our work suggests these disorders occur more frequently than believed. Ongoing work includes patient recall for confirmatory genetic testing and clinical evaluation.

W155. Systemic Assessment of Obesity as a Causal Risk Factor for Immune and Inflammatory Diseases

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Abstract Text: Obesity represents a growing global health concern, with emerging evidence suggesting links to immune and inflammatory (I&I) diseases beyond its established metabolic consequences. However, the directionality and potential causal nature of these relationships remain unclear. To identify novel mechanisms driving the obesity-I&I axis, we investigated the relationship between obesity and 3,410 disease endpoints using longitudinal data from FinnGen, a biobank project linking genetic and health information across 500K individuals. Cox proportional hazard models identified 316 associations between obesity and disease endpoints, with obesity

increasing risk in 96% of cases. Beyond expected associations with diabetes (HR=3.8, P< 1×10^{-300}) and cardiometabolic conditions like hypertensive heart disease (HR=4.0, P=6×10^{-238}), obesity strongly correlated with I&I diseases including hidradenitis suppurativa (HR=4.9, P=2×10^{-15}), psoriasis (HR=4.3, P=2×10^{-16}), and asthma (HR=1.8, P=2×10^{-47}). To establish causality, we tested associations between genetically predicted BMI, derived from independent polygenic score weights, and disease outcomes. This approach revealed 820 significant endpoints, including hidradenitis suppurativa (OR per 1SD=1.34, P=2×10^{-27}), early-onset COPD (OR=1.17, P=4×10^{-95}), and psoriasis vulgaris (OR=1.16, P=2×10^{-8}). Among 165 I&I-related endpoints, 69 showed positive associations, predominantly in rheumatologic, respiratory, and skin disorders. We then assessed reverse associations, that is I&I diseases increasing risk of obesity, by testing the associations between I&I disease polygenic scores and obesity risk. These relationships were absent or weak, with asthma showing the strongest effect (OR=1.06, P=2×10^{-22}). Our findings support obesity as a potential causal risk factor for I&I diseases, but not vice versa. Notably, hidradenitis suppurativa showed the strongest associations with obesity, comparable to diabetes and cardiometabolic diseases.

W156. The Broad Clinical Spectrum of IRF4 p.T95R Combined Immunodeficiency

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Abstract Text: Damaging interferon regulatory factor (IRF) variants cause a unique subset of inborn errors of immunity (IEIs), termed inborn errors of IRFs. Phenotypes vary from combined immunodeficiency to craniofacial anomalies, with IRF4 deficiencies causing immune impairment. Of IRF4-associated disorders, the IRF4 p.T95R has the most severe phenotype. We expand the clinical profile of nine patients, with a focus on hematopoietic stem cell transplant (HSCT) outcomes. Key clinical features for all nine IRF4 p.T95R patients, including two new patients, were extracted from clinical records, publications, and clinician follow-up. At reporting, 7/9 patients are living, aged 6.5 to 31 years. All have combined immunodeficiency with impaired B-cell development and impaired antibody production. Each presented with opportunistic infections in their first year of life, including Pneumocystis jirovecii (8/9), cytomegalovirus (3/9), onychomycosis (3/9), and atypical mycobacteria (2/9). Gastrointestinal manifestations were present in 5/9 patients. All were managed with immunoglobulin replacement therapy and antimicrobial prophylaxis. 4/9 patients were treated with HSCT. 2/4 died from infectious complications (pretransplant CMV and cryptosporidium), 1/4 lost their graft, and 1/4 had a positive outcome with protection from infections and resolution of enteropathy. 2/9 patients survived until adulthood without HSCT. Outcomes varied across IRF4 p.T95R patients. While all had profound combined immunodeficiency, two reached adulthood without HSCT, and four required HSCT in childhood. The mixed HSCT outcomes in our cohort underscore the importance of early intervention, before infection. Compared to other IRF4-associated IEIs, IRF4 p.T95R patients have a uniquely severe phenotype, reinforcing the need to consider genotype-specific effects in IEI patients.

W157. Targeted Deep Sequencing Identifies Mosaicism in Patients with Immune Dysregulation

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Abstract Text: Identifying genetic mechanisms of inborn errors of immunity (IEI) is important for diagnosis and treatment of patients, yet most patients with suspected IEI have negative genetic testing. Mosaicism is an emerging genetic mechanism of IEI that is difficult to identify due to the low frequency of variants and limited depth of exome and genome sequencing. This study aims to identify mosaic variants in immune-related genes in patients and healthy cohorts. We developed custom panels for high-depth sequencing of genes known or hypothesized to cause dominant immune dysregulation. Samples from 452 patients with immune dysregulation (affected) and 154 currently healthy individuals were sequenced using either a 71 gene (average depth 6105x) or 101 gene (average depth 3182x) targeted panel. A custom bioinformatics pipeline utilizing the union of 3 variant callers (Mutect, Varscan, and DoCM) was used to identify mosaic variants with variant allele fractions (VAF) as low as 1%. We validated this pipeline using both resesquencing an independent library and droplet digital PCR. We identified mosaic variants in 9.5% of undiagnosed patients and 7.8% of healthy individuals with an average VAF of 4.4% (range 1.0%-24.2%). 54.2% of variants in affected (26/48) and 25% of variants in healthy individuals (3/12) were predicted to modify the sequence of the encoded protein. Using a strategy to predict pathogenicity of variants in IEI, 33% of variants identified in patients were predicted to be likely pathogenic or pathogenic, while no mosaic variants in healthy individuals were predicted to be pathogenic. We identified mosaic variants in 34 genes with 25/34 genes not previously known to cause mosaic GEI. Genes with mosaicism in >1 affected patient included: FAS, STAT3, CARD11,CARD14, NRAS, TNFAIP3, NLRP3, and IKZF2. Four patients had FAS variants with allele fractions < 5% in blood but highly enriched in double-negative T cells, diagnostic for somatic FAS autoimmune lymphoproliferative syndrome. These findings establish the utility of a high-depth sequencing panel to identify pathogenic mosaic variants and demonstrate that mosaicism in immune-relevant genes is present in healthy individuals. It also establishes a strategy to identify and classify mosaic variants in the context of IEI.

W158. Primary Amoebic Meningoencephalitis Caused by Complement C2 Deficiency

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Abstract Text: Primary amoebic meningoencephalitis (PAM) is a rapidly progressive and often fatal central nervous system infection caused by Naegleria fowleri (aka. Brain-eating amoeba). With a mortality of >95% regardless of treatment, PAM is one of the most lethal infectious diseases known. Despite widespread environmental exposure to N. fowleri, PAM is rare, suggesting that an undiagnosed rare genetic disease may underlie it. There are only 11 PAM survivors reported in the scientific literature. We studied a previously healthy 10-year-old girl who was diagnosed with PAM and survived following treatment, representing an additional documented survivor that enabled detailed immunologic and genetic investigation. Our results show this patient has normal frequencies of all immune cell subsets tested, but carries a homozygous deletion in C2, resulting in complete complement component 2 (C2) deficiency and abolishing classical complement pathway activity. We show that normal human serum induces complement-mediated lysis of N. fowleri *in vitro*. In contrast, C2-depleted serum and serum from the C2-deficient patient neither kill the parasite nor deposit membrane attack complex (MAC) on its surface. Both MAC deposition and amoebicidal activity are restored in the patient serum upon supplementation with purified human C2. These findings demonstrate that loss of classical complement pathway activity due to a monogenetic defect is sufficient to cause PAM.

W159. Leveraging Diverse Biobanks to Improve Genetic Discovery in Sepsis

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Abstract Text: Background: Sepsis is a severe systemic inflammatory response triggered by infection, contributing to ~11 million deaths worldwide. Although genetics studies suggest a heritable component to sepsis susceptibility, variants discovered through genome-wide association studies (GWAS) show poor replication across cohorts, hindering their utility for advancing understanding of sepsis biology and improving clinical care. Here, we leverage three large diverse biobanks to investigate sources of discordance in sepsis GWAS and identify novel genetic risk factors. Methods: We conducted ancestry-stratified GWAS (MAF ≥0.5%) in All of Us (AoU) version 7 & 8, BioMe, and UK Biobank using a generalized linear mixed model implemented in SAIGE, accounting for age, sex, and genotyping principal components. We meta-analyzed the results using inverse-variance weighting via METAL (N total ~720,000; ~33,000 cases, defined using PhecodeX ID_092.2: 80% European, 10% African, 8% Admixed American, 1% South & East Asian). To evaluate the effect of phenotyping, in AoUv7 we performed GWAS using Sepsis-3 clinical-diagnostic criteria and compared against PhecodeX ID_092.2. Results: The Sepsis-3 and PhecodeX GWAS showed correlation in overall Z-scores (Pearson's r =0.79, p< 2.2x10-16) and strong correlation (Pearson's r =0.96, p< 2.2x10-16) in SNPs reaching suggestive significance (p< 1x10-5). This indicates a shared genetic architecture and that phenotyping using PhecodeX can provide a scalable and reliable approach for identifying sepsis cases in large biobanks. Meta-analysis across biobanks identified 6 and 602 SNPs reaching genome-wide (p< 5x10-8) and suggestive significance, respectively. We observed low heterogeneity (96% of SNPs had Cochran's p >0.05) and no genomic inflation (lambda = 1.03). Among the suggestive SNPs, 5 were exonic, 133 intronic, 348 intergenic, and 122 were other noncoding variants. Most notably African ancestry-specific rs334 was significantly associated with increased risk of sepsis (beta = 0.33, S.E. = 0.058, p< 4x10-8). This missense variant causes sickle-cell anemia (SCA) in homozygous. Our results suggest that contrary to common wisdom, carriers of

SCA (>100 million worldwide) are at a higher risk of sepsis, indicating that screening for carrier status may contribute to reducing sepsis burden. Future work will focus on fine-mapping and functional genomics analysis to understand variant function and gain insight into sepsis biology.

W160. T Cell Intrinsic NOD2 Mutations Drive IL-17-mediated Inflammatory Disease in Blau Syndrome

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Abstract Text: Objectives Blau syndrome is a rare early-onset granulomatous inflammatory disease caused by mutations in NOD2 and has traditionally been classified as an autoinflammatory disorder driven by innate immune dysfunction. However, emerging evidence suggests that Blau-associated NOD2 variants impair microbial sensing and may instead promote maladaptive adaptive immune responses. Building on our prior identification of a T cell–intrinsic role for NOD2 in restraining IL-17 production, this study aimed to define the contribution of Th17 immunity to Blau pathogenesis and to determine whether IL-17 represents a therapeutically actionable pathway in this disease. Methods Peripheral blood mononuclear cells (PBMCs) from patients with Blau syndrome harboring six distinct NOD2 mutations (ages 2–64, n=8) and healthy controls (n=10) were analyzed *ex vivo* and following stimulation with anti-CD3/anti-CD28 or PMA/ionomycin. Immune cell composition, T cell transcription factors, and cytokine production were quantified by multiparameter flow cytometry. For clinical translation, two treatment-refractory Blau patients received IL-17–targeting biologics (secukinumab or bimekizumab) and were followed longitudinally. Patients were treated with IL-17 inhibitors and clinical outcomes were assessed using validated patient-physician reported disease activity and functional questionnaires at baseline and post-treatment, alongside longitudinal immune profiling and serum biomarker analysis. Results Across genetically diverse Blau patients, we observed a consistent enrichment of IL-17-producing $CD4^+$ T cells compared with healthy controls. Blau PBMCs exhibited increased frequencies of $ROR\gamma t^+$ Th17 cells and heightened production of IL-17A and IL-17F following T cell receptor stimulation, with expansion of polyfunctional Th17 populations co-expressing IL-17A, IL-17F, and TNF and a notable bias toward IL-17F. IL-17 blockade in two refractory patients led to rapid and marked clinical improvement within three weeks, including reduced pain and functional disability. These responses were accompanied by profound immunologic changes, including reductions in circulating $CD3^+CD4^+$ T cells, $ROR\gamma t^+$ Th17 cells, Th17-associated cytokines (IL-17, GM-CSF, TNF), and normalization of elevated soluble IL-2 receptor levels. Conclusions These findings identify dysregulated Th17 immunity as a central feature of Blau syndrome and provide the first evidence that IL-17 signaling is both elevated and therapeutically actionable. This work reframes Blau syndrome as a NOD2-driven, T cell–mediated inflammatory disease and supports targeted IL-17 inhibition as a rational treatment strategy.

W161. 3D Regulatory Mapping of Human ILC3s Resolves Cell Type-specific Autoimmune Risk

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Abstract Text: Type 3 innate lymphoid cells (ILC3s) promote mucosal barrier integrity but can also amplify intestinal inflammation. Linking autoimmune GWAS signals to effector genes remains challenging because most variants are non-coding and act through cell type-specific regulatory elements, particularly in rare populations such as ILC3s. Because many risk variants reside in distal enhancers, resolving three-dimensional chromatin contacts is essential for assigning variants to their target genes. We therefore generated high-resolution, low-input promoter capture Hi-C maps in primary human ILC3s and matched CD4 T cells, defining >60,000 promoter-linked chromosomal contacts across both cell types. Promoter-interacting regulatory elements in both ILC3s and CD4 T cells were significantly

enriched for autoimmune risk variants. Using Crohn's disease as a case study, we integrated promoter contacts with regulatory activity and GWAS fine-mapping to prioritize candidate effector genes, identifying 109 risk genes in ILC3s and 118 in CD4 T cells. This approach revealed shared lymphocyte regulatory circuitry while also highlighting candidates selectively implicated in ILC3 biology. One notable ILC3-specific candidate was Cln3, a lysosomal gene known for causing the neurodegenerative disorder Batten disease yet largely unstudied in the immune system. In mouse ILC3-like cells and primary human ILC3s, Cln3 expression decreased following inflammatory stimulation. Preventing this downregulation via CRISPR activation or ectopic overexpression blunted activation-associated transcriptional programs and reduced secretion of IL-17, IL-22, and GM-CSF. These data indicate that lysosomal pathways may function as a cell-intrinsic brake on ILC3 inflammatory output. Extending this framework across six autoimmune diseases revealed consistent enrichment of prioritized risk genes involved in regulating ILC3 inflammatory responses. Together, these findings define the three-dimensional regulatory architecture of human ILC3s and establish a generalizable strategy for translating non-coding genetic risk into cell type–specific, testable mechanisms underlying autoimmune disease.

Immunology Across the Ages

Tu158. Distinct Metabolic Profiles of Early-life Naïve $CD8^+$ and $CD4^+$ T Cells After Activation

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Abstract Text: Neonatal immune responses differ markedly from those of adults, partly due to distinct metabolic programs within immune cells. Because cellular function is tightly coupled to metabolic state, defining these early-life programs is essential for understanding age-specific immunity. We previously showed that isolated neonatal naïve $CD8^+$ T cells have greater glycolytic capacity and enhanced TNF-alpha production after activation compared to their adult counterparts. However, it remains unknown whether these differences persist when T cells are evaluated directly in the more physiological context of whole blood, where multiple cell types and signaling networks interact. We compared cellular reliance on glycolytic versus oxidative pathways for protein synthesis of neonatal, < 2-year-old child, and adult naïve $CD8^+$ and $CD4^+$ T cells using the SCENITH (Single-Cell ENergetIc metabolism by profiling Translation InHibition) assay in PMA/ionomycin-stimulated, RBC-lysed whole blood. In parallel, we measured the expression of transcription factors and effector molecules to link the metabolic state to the functional phenotype. We found that neonatal activated naïve $CD8^+$ and $CD4^+$ T cells exhibit significantly lower mitochondrial dependence and higher glycolytic capacity compared to their adult counterparts. Notably, this metabolic phenotype persists in activated naïve $CD8^+$ and $CD4^+$ T cells from healthy children up to two years of age, and from preliminary results, we have observed a similar phenotype in this age group in children with severe RSV bronchiolitis. Additionally, neonatal $CD4^+$ T cells show reduced glucose dependence and increased capacity to utilize fatty acids and amino acids for ATP generation. Lastly, we demonstrate that within the whole-blood context, previously observed differences in effector molecule production by isolated and activated neonatal naïve $CD8^+$ T cells are attenuated, suggesting modulation by extrinsic cellular or soluble factors. In conclusion, early-life T cells demonstrate enhanced glycolytic capacity and greater flexibility in substrate utilization, while effector function is shaped by the surrounding immune environment. Future efforts will focus on defining the cell-intrinsic signaling pathways and extrinsic soluble factors that drive these age-specific energetic profiles. Defining these age-specific metabolic programs will help distinguish normal developmental immune trajectories from pathogenic immune responses and may inform the development of age-tailored immunotherapeutic strategies.

Tu159. Impaired Adaptive Senescence in Fibro/Adipogenic Progenitors Contributes to Exercise Resistance in Type 2 Diabetes

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Abstract Text: Exercise is widely recognized as an effective intervention for obesity and diabetes, offering numerous health benefits. Nevertheless, it is documented that approximately 15–20% of individuals with Type 2 Diabetes (T2D) fail to respond to exercise therapy. While inflammation acts as a beneficial adaptive signal for myogenesis, exercise-induced inflammatory profiles differ significantly between Normal Glucose Tolerance (NGT) and T2D. We hypothesized that this discrepancy stems from dysregulated cellular senescence and its associated inflammatory program, the Senescence-Associated Secretory Phenotype (SASP), which is essential for facilitating muscle repair. Cellular senescence is an adaptive stress response characterized by irreversible cell cycle arrest and SASP. While the role of senescence in skeletal muscle remains a subject of debate, emerging consensus highlights its critical function in tissue remodeling. Specifically, we previously demonstrated that exercise-induced muscle damage triggers senescence in fibro/adipogenic progenitors (FAPs) (Nat Commun, 2020), facilitating muscle repair via SASP. However, we postulate that in the chronic inflammatory state of T2D, FAPs fail to undergo this requisite senescence. First, we performed a Gene Set Enrichment Analysis and meta-analysis of human skeletal muscle transcriptomics (GSE59363 and MetaMEx). We found that exercise significantly upregulated senescence-related genes in NGT subjects; however, this adaptive response was attenuated in T2D muscles. Next, to identify the specific cell types involved, we re-analyzed murine single-cell RNA sequencing data (GSE183288). In normal mice, exercise induced a specific FAP subcluster, termed "Senescent FAPs," characterized by enhanced cell-cell communication with macrophages and muscle stem cells. In contrast, FAPs from High-Fat Diet mice failed to exhibit this senescence phenotype; instead, they showed upregulation of inflammation- and fibrosis-related signals. Finally, we examined these responses *in vitro* using FAP-like TIG-2M cells. Mechanical stress increased senescence markers under normal conditions. However, in a diabetic model(high glucose and TNF-α), this senescence induction was inhibited. Furthermore, the inflammation marker NF-κB was significantly upregulated under diabetic conditions, suggesting a shift from adaptive senescence to chronic inflammation. In conclusion, our findings suggest that the attenuation of adaptive senescence in FAPs contributes to the reduced efficacy of exercise in T2D skeletal muscles.

W162. Age-related Changes in Lymphoid and Lung Tissues Revealed by Automated, In-depth Mouse Immune Profiling with CyTOF Technology

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Abstract Text: Introduction/Rationale Immunosenescence affects immune surveillance and leads to age-related diseases, but its mechanisms are not fully understood. Using CyTOF™ technology and the Maxpar™ OnDemand Mouse Immune Profiling Panel Kit with Maxpar Pathsetter software, this study profiles immune changes in spleen and lung tissues of young and aged mice. Methods C57BL/6J mice (aged 75–76 weeks and 6–8 weeks) were used. Spleen and lung single-cell suspensions were stained with 33- and 50-antibody panels, respectively. Live-cell CD45 barcoding allowed pooled analysis. Data was collected using a Helios™ system and analyzed with Maxpar Pathsetter, using a pre-designed analysis template, to automatically identify 38 splenic and 55 lung immune subsets. Results In the spleen, aged mice demonstrated decreased frequencies of CD4 and CD8 T cells, NK cells and γδ T cells, accompanied by an increase in PD-1$^+$ T cells and B cells. NK cell maturation was disrupted, as evidenced by altered expression profiles of PD-L1, CD11c and CD49b. In the lungs, aged mice presented elevated levels of exhausted CD8 T cells (PD-1$^+$TIM-3$^+$), cytotoxic T cells (granzyme B$^+$) and monocyte-derived dendritic cells, in addition to a pro-inflammatory phenotype in alveolar macrophages. In total, 26 splenic and 28 pulmonary cell subsets exhibited significant age-related changes. Conclusion CyTOF immune profiling with automated analysis

enables detection of age-related immune changes. Using the Mouse Immune Profiling Panel and Maxpar Pathsetter software provides a scalable method for detailed mouse model analysis. This strategy supports research on immunosenescence and translational studies in aging and immune modulation. For Research Use Only. Not for use in diagnostic procedures.

W163. Evidence of Activation and Immune Modulation During Wildfire Smoke Exposure in Healthy Adults Mediated by Age

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Abstract Text: The incidence of severe air pollution due to wildfire smoke is increasing in the United States; however, most research has focused on long-term, sustained exposures rather than acute, extreme events. The immunological effects of acute smoke exposure remain poorly understood, particularly with respect to age-related differences. Objective: To characterize the acute immunological effects of wildfire smoke exposure in younger and older healthy adults. Methods: In 2020, the Pacific Northwest experienced severe wildfire smoke, with air quality index values reaching unhealthy and very unhealthy levels for weeks. To assess the acute effects of wildfire smoke we utilized data from the Sound Life Project (SLP), a cohort of healthy adults followed longitudinally over two years, completing 10 study visits. At each visit, blood samples were collected and immunophenotyped using mass cytometry. We identified 48 participants with samples collected during or shortly after this wildfire smoke event, when changes in immune cell populations could be expected. Each participant had an additional comparison visit in either 2019 or 2021 during the same season (August – October), when air quality was good to moderate. For each age group, we conducted paired analysis of visits during and outside a wildfire smoke event to assess differences in circulating immune cell populations. Results: Out of 47 immune cell populations assessed, 16 were significantly altered with acute wildfire smoke exposure in both the younger and older age groups. These included increases in multiple activated T cell populations, regulatory T cells, PD-L1 expressing dendritic cells, monocytes, and B cells. Naive $CD8^+$ T cell populations were uniquely increased in the younger adults, as were $CD38^+$ $CD4^+$ T cells. $CD69^+$ $CD4^+$ and $CD69^+$ $CD8^+$ T cell populations were uniquely decreased in the older adults. Conclusions: Acute wildfire smoke exposure was associated with measurable differences in the frequencies of immune cells, especially activated T cells. These effects varied by age, with some activated T cell populations uniquely decreasing in older adults. In future work, we plan to expand this analysis to transcriptional and proteomic datasets available for this cohort.

Immunometabolism

Th165. Increased Glutamine Dependence of CD8 T Cells in Human Critical Illness Drives Dysregulated Effector and Exhausted-like Programming and Associates with Poor Clinical Outcomes

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Abstract Text: Bioenergetic dysfunction and acquired immunosuppression are hallmarks of critical illness syndromes including sepsis. CD8 T cell impairment has been linked to increased mortality and more severe organ dysfunction in ICU patients. However, whether metabolic mechanisms underlie CD8 T cell defects remains poorly understood. To answer this, we performed an integrated set of *ex vivo* and in silico experiments using human

peripheral blood mononuclear cells (PBMCs) collected from medical and surgical ICU patients at Vanderbilt University Medical Center and age- and sex-matched healthy controls. Using functional metabolic profiling by flow cytometry (SCENITH), we found that CD8 T effector/effector memory (EM) cells from critically ill (CI) patients were more dependent on glutamine for overall metabolic output compared to EM cells from healthy control (HC) participants (8.8 FC, p=0.019). To validate this finding, we analyzed our single-cell RNA-sequencing dataset of PBMCs from 38 ICU and 9 HC participants. Consistent with our SCENITH results, in silico flux balance analysis via Compass demonstrated CI CD8 T EM cells had increased predicted flux through glutaminase (GLS), which catabolizes glutamine to glutamate. To assess whether glutamine causally drives dysfunction, we restimulated PBMCs in the presence of the glutaminase inhibitor CB-839 and performed immunophenotyping by high-dimensional spectral flow cytometry. CI CD8 T cells at baseline displayed a dysfunctional state characterized by concurrent elevation of exhaustion-like programming (TOX, p=0.001; PD-1$^+$TIM-3$^+$ co-expression, p< 0.001), effector programming (T-Bet, p< 0.001), activation markers (CD38, p< 0.001; CD25, p=0.001), and mitochondrial stress (Cytochrome C, p=0.006). However, glutaminase inhibition partially rescued this dysfunctional state, with reductions in TOX (p< 0.001), PD-1$^+$TIM-3$^+$ co-expression (p=0.006), T-Bet (p< 0.001), CD38 (p< 0.001), CD25 (p< 0.001), and Cytochrome C (p< 0.001). Notably, patients with evidence of severe circulatory dysfunction (plasma lactate >2) or who died in-hospital exhibited the highest CD8 T EM glutamine dependence as determined by SCENITH (p=0.035 and p=0.066, respectively). Additionally, CI patients with lactate >2 trended toward larger TOX reductions with CB-839 (p=0.099). Our findings identify glutamine as a central metabolic node sustaining CD8 T cell dysfunction in critical illness and suggest interventions targeting glutamine pathways merit investigation for restoring adaptive immunity in ICU patients.

Th166. NQO1-mediated Immunometabolic Reprogramming by LMT503 Restores T Cell Plasticity and Restrains Neutrophil Pathogenicity in Colitis

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Abstract Text: BACKGROUND: Inflammatory bowel disease (IBD) is driven by dysregulated crosstalk between adaptive and innate immune cells. While LMT503 is known to activate NAD(P)H quinone dehydrogenase 1 (NQO1), its capacity to coordinate both adaptive and innate immune responses in the context of mucosal homeostasis remains unclear. We hypothesized that pharmacological NQO1 activation would resolve intestinal inflammation by coordinately modulating T cell plasticity and neutrophil effector functions. METHODS: Therapeutic efficacy of LMT503 was assessed in an adoptive T cell transfer-induced colitis model and compared with the JAK inhibitor tofacitinib. Mechanistic studies were performed in human primary CD4$^+$ T cells, CD66b$^+$ neutrophils, and NQO1 knockdown systems using flow cytometry, transcriptional profiling, mitochondrial functional analyses, and imaging-based assays. RESULTS: LMT503 significantly ameliorated experimental colitis, reducing histologic inflammation and attenuating colon shortening. Notably, in the intestinal lamina propria, LMT503 suppressed pathogenic Th1 and Th17 cell infiltration while robustly expanding Foxp3$^+$ regulatory T cells (Treg) in a dose-dependent manner, a restorative effect not observed with tofacitinib. LMT503 also reduced systemic inflammatory cytokines while inducing colonic expression of Nqo1 and Ppargc1a. In T cells, LMT503 enhanced mitochondrial remodeling and oxidative metabolic signatures while promoting Treg differentiation. LMT503 augmented IL-2-induced STAT5 phosphorylation and AMPK activation under glycolytic inhibition, whereas tofacitinib suppressed both pathways. Importantly, NQO1 knockdown abrogated LMT503-mediated induction of p-STAT5 and p-AMPK, establishing NQO1-dependent link between metabolic sensing and cytokine signaling. In neutrophils, LMT503 reduced cytosolic and mitochondrial reactive oxygen species and suppressed NETosis, while preserving antimicrobial-associated markers CD177 and IL-22, demonstrating selective modulation of pathogenic effector programs without compromising host defense capacity. CONCLUSIONS: These findings identify NQO1 activation as a metabolic chekpoint that governs T cell plasticity and innate cell pathogenicity. LMT503-mediated NQO1 activation represents a promising immunometabolic strategy for IBD, by suppressing inflammation while promoting mucosal immune tolerance.

Tu160. LL-37 Induces Inflammatory Signatures on B Cells via Unsaturated Fatty Acid Metabolism in Psoriatic Arthritis

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Abstract Text: 【Background】Antimicrobial peptides are involved in the pathogenesis of psoriatic arthritis (PsA). LL-37 is elevated in PsA synovial fluid and is known to activate dendritic cells and T cells, but its effects on B cells remain unclear. We investigated whether LL-37 induces inflammatory programs in B cells and explored the involvement of unsaturated fatty acid metabolism. 【Methods】Public single-cell RNA sequencing (scRNA-seq) data from synovial tissues of patients with rheumatoid arthritis (RA) and PsA were analyzed to characterize B cell transcriptional profiles. Human peripheral blood $CD20^{+}CD27^{+}$ memory B cells were purified and stimulated with CpG and LL-37. TNF and IL-6 in culture supernatants were quantified by ELISA. Transcriptomic changes after LL-37 stimulation were assessed by bulk RNA-seq. To test metabolic dependency, memory B cells were treated with the stearoyl-CoA desaturase (SCD) inhibitor A939572 and inflammatory cytokine production was evaluated. 【Results】4,249 B cells were identified in synovial tissues. Pathway enrichment in PsA B cell clusters showed stronger detection of inflammatory cytokine signaling, including TNF and IL-17–related pathways, compared with RA. Memory B cells treated with CpG and LL-37 significantly increased TNF and IL-6 production. Bulk RNA-seq of LL-37–stimulated memory B cells revealed significant upregulation of enzymes involved in unsaturated fatty acid metabolism, including SCD and FADS1. Pharmacologic inhibition of SCD with A939572 suppressed LL-37–associated inflammatory cytokine production. 【Conclusion】LL-37 promotes inflammatory activation of human memory B cells and induces unsaturated fatty acid metabolic programs. These findings suggest that LL-37 may amplify PsA inflammation through B cell–intrinsic metabolic rewiring, and that targeting lipid desaturation pathways could represent a therapeutic strategy to modulate B cell–driven cytokine responses in PsA.

Tu161. Immune Proteomic Landscape of Alcoholic Liver Disease Progression and Abstinence-associated Modulation

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Abstract Text: Olink Bioscience's Proximity Extension Assay (PEA, Uppsala, Sweden) enables multiplexed profiling analysis of immune mediators (cytokines, chemokines, growth factors) involved in chronic inflammatory diseases. The Olink Target 48 Cytokine panel (Olink-48) provides both absolute concentrations (QUANT, pg/mL) and relative expression values (Normalized Protein eXpression or NPX) for cytokines and related proteins, while the Immuno-Oncology 96 panel (Olink-96) reports only NPX values. This study aimed to evaluate the correlation between NPX values from the Olink-96 panel with absolute quantitative values from the Olink-48 panel in patients with alcoholic liver disease (ALD). A total of 37 serum samples, including 3 from healthy controls and 34 from 17 ALD patients, were analysed using both panels. Thirty overlapping proteins were identified based on matching UniProt IDs (unique protein identifiers). Spearman correlation coefficients were calculated, and quality control (QC) metrics were evaluated. Protein-level analysis based on UniProt IDs revealed a strong positive correlation between Olink-48 QUANT values and Olink-96 NPX values (mean rho = 0.81, $P < 0.05$). Proteins such as IL-6, IL-7, CXCL10, and EGF showed particularly high concordance (rho = 0.99), while IL-2, IL-13, IL-33, and IL-4 showed weak correlations (rho = –0.02 to 0.22), associated with low QC validity in the Olink-96. At the individual sample level, strong correlations were observed between Olink-48 NPX and Olink-96 NPX (mean rho = 0.93, $P < 0.05$), and between Olink-48 QUANT and Olink-96 NPX (mean rho = 0.81, $P < 0.05$). NPX data from the Olink-96 panel shows high concordance with absolute quantification from the Olink-48 panel, suggesting that NPX can serve as a comparative indicator even in quantitative biomarker studies. However, caution is advised when interpreting markers with low QC performance.

Tu162. Scavenger Receptor MARCO: A Novel Regulator of Macrophage Senescence and Therapeutic Target in Cancer Progression

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Abstract Text: Scavenger Receptors (SRs) are a functionally diverse, multi-ligand family of pattern recognition receptors (PRRs) primarily expressed on myeloid cells. Involved in both innate immunity and homeostasis, dysregulation of SRs is associated with numerous infectious, inflammatory, metabolic, and malignant pathologies. However, a unifying mechanism is currently lacking for such functional diversity. Here, we demonstrate that loss of macrophage receptor with collagenous structure (MARCO) is linked to cellular senescence, a contributor to many such pathologies. MARCO knockout (MKO) macrophages demonstrated increased cellular volume and ~3-fold decreased doubling time compared to wildtype (WT) macrophages in CFSE-based flow cytometric analysis. A basal "senescent-like" MKO phenotype was further observed *in vitro* comprising altered Lamin B1 protein levels, increased p21 mRNA and protein expression, and increased β-galactosidase staining compared to WT macrophages. This differential in senescence markers between MKO and WT macrophages increased following 5-day treatment with 1 μM of cisplatin or chamber-induced hypoxia. Potential impact of these findings are highlighted by our *in vitro* co-culture model in which WT macrophages induced a subset of highly proliferative HGS2 ovarian cancer cells (HPHCs) that were absent in HGS2 monoculture, whereas HGS2-MKO co-cultures demonstrated substantially reduced HPHC populations. Strikingly, HPHC populations were virtually abolished in trans-well co-culture with both WT and MKO macrophages, suggesting their induction is contact-dependent. Blocking MARCO with an antibody-like "Ankyron" protein in WT macrophages partially recapitulated the reduced HPHC populations observed with MKO macrophages. *In vivo*, anti-MARCO Ankyron administration likewise reduced HGS2 tumour volume by ~10% compared to control mice. How knockout of MARCO enhances senescent-like cellular features whilst conversely reducing macrophage-induced effects on cancer cell proliferation remains a key question. However, collectively our work implicates this SR in both processes and provides the rationale for therapeutic targeting of MARCO in ageing and age-related diseases such as cancer.

Immuno-oncology

Th125. Implementation of a Novel Immune Monitoring Strategy for Multicenter Clinical Trials to Enable Equity in Cancer Prognosis for Individuals from Remote Settings of Australia

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Abstract Text: Executing longitudinal immune phenotyping studies by flow cytometry is complex, primarily due to logistical challenges that could affect data quality. This is exacerbated when considering remote and rural communities in Australia, which are burdened by both an increased prevalence and worse prognosis of many chronic and infectious pathologies. This is not unique to the Australian context, as global remote and rural communities are underrepresented in clinical trials. Recent developments in CyTOF technology facilitate a highly simplified workflow of asynchronous sample collection and staining, compatible with current hospital laboratories, which typically run on unpredictable schedules, to remote settings, which are resource-poor and have limited clinical trial infrastructure. This is uniquely enabled by using a stable dried-down cocktail of CyTOF antibodies in an easy-to-follow protocol that yields reproducible staining while minimizing sample required. Furthermore, this workflow, unique

to mass cytometry, provides flexibility and minimizes technical variation via sample barcoding and freezing of stained samples for shipment to a central site for batch acquisition. Previously, our group developed a novel blood-based "immune signature" that robustly predicts clinical response in PD-1/PD-L1-targeted checkpoint therapies in melanoma and lung cancer. The current study is designed to demonstrate both clinical impact of the large panel and utility of CyTOF technology for a highly simplified and robust 50-plus parameter immune profiling workflow for multi-site clinical trials. Here, we present findings from a 100-sample pilot study at three sites across Australia in a variety of clinical implementation setups. Remote asynchronous sample collection was performed to compare two surface staining approaches on i) PBMC versus ii) plasma-depleted whole blood to suit settings where centrifugation access was readily available or not, respectively. Stained samples were shipped to a central lab for further processing. In the case of cryopreserved whole blood, bead-based granulocyte depletion was first performed, with all samples subsequently multiplex barcoded together for intracellular antibody staining of key functional markers. Analysis harmonization was achieved across the sample collection sites and staining approaches tested. Overall, this workflow enables access to underserved communities, facilitating for the first time equity and scalability of immune phenotyping studies to harness truly geographically dispersed clinical centers.

Th167. Evaluating Busulfan and Anti-GPIbα Platelet Depletion Strategies in ApcMin/$^{+}$ Mouse Models of Cancer Cachexia

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Abstract Text: Cachexia is a debilitating muscle-wasting syndrome associated with chronic illnesses including cancer, AIDS, heart failure, and chronic kidney disease. Although inflammation is a key driver of cachexia, the specific roles of individual immune components remain unclear. Platelets, anucleate cell fragments derived from megakaryocytes, are increasingly recognized as important regulators of inflammation and immunity. We previously found that platelet counts rise before cachexia onset in ApcMin/$^{+}$ mice and that platelets become activated as the condition progresses. In this study, we investigated the effects of platelet depletion in cancer-associated cachexia using the ApcMin/$^{+}$ mouse, a well-established model of spontaneous colorectal cancer and cachexia. We profiled circulating immune cells across disease stages and employed two platelet-depletion strategies: a monoclonal anti-GPIbα antibody and the chemotherapeutic agent busulfan. Functional outcomes were assessed through grip-strength testing, muscle-mass measurements, and hematological and immunological analyses. Anti-GPIbα treatment selectively reduced resting platelet numbers while leaving activated platelets unchanged. Unexpectedly, busulfan increased platelet counts exclusively in ApcMin/$^{+}$ mice, counter to its anticipated depleting effect in both ApcMin/$^{+}$ and WT animals. These measurements showed that platelet phenotype more accurately distinguished cachectic from pre-cachectic mice compared to blood or muscle profiles. In summary, these findings indicate that platelets contribute to immune regulation in cancer cachexia and highlight their possible function as muscle-wasting sensors and potential biomarkers of cachexia progression.

Th168. A Common Pretreatment Immune Setpoint Predicts Autoimmune Toxicity Across Cancers, Organs, and Checkpoint Inhibitors

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Abstract Text: Objectives: Immune checkpoint inhibitors improve survival in several malignancies but can cause immune related adverse events (irAEs)–severe autoimmunity requiring treatment discontinuation in >20% of patients. Clinical guidelines highlight the need for biomarkers to quantify baseline irAE risk and identify immune pathways that can be targeted without compromising antitumor immunity. Our objectives were to: 1) Define pre- and post-treatment blood immune phenotypes associated with irAE development. 2) Identify irAE-associated states independent of antitumor response, including within tumor-infiltrating lymphocytes (TILs). 3) Test whether irAE-specific immune states predict severe irAEs across organs and checkpoint inhibitor classes. Methods: We performed CITE-seq on blood from avelumab-treated thymic epithelial tumor patients; all achieved antitumor responses, and half developed grade ≥3 myositis irAEs. Mixed-effects models identified pretreatment differences between irAE and non-irAE patients that were independent of treatment effects. We then built an atlas of ~400,000 TILs integrated with irAE-lesion datasets to remove ICI-induced TIL programs and retain phenotypes upregulated in irAE target tissues. Finally, we trained a random forest model to predict irAEs from baseline bulk blood gene expression in external cohorts using TIL-independent genes. Results: Patients who developed irAEs exhibited a longitudinally stable immune setpoint with correlated baseline transcriptional activity across inflammatory monocytes, $CD1c^+$ dendritic cells and mTORC1-activated $CD8^+$ TEMRA/effector subsets. These programs were not induced in TILs but were selectively amplified in inflamed irAE tissues across independent datasets. A blood transcriptional predictor derived from TIL-independent genes predicted severe irAEs (AUC=0.74, held-out test set) spanning eight organ systems in external validation across n=561 patients from multiple countries treated with multiple different ICIs. Conclusions: We identified a baseline immune setpoint coupling inflammatory and mTOR signaling that predicts severe irAEs across organs and cancers while remaining independent of ICI-driven TIL programs. Our blood surrogate underpins ongoing efforts to develop clinically deployable assays for baseline irAE risk stratification and prioritization of patients for targeted irAE therapies that preserve antitumor immunity. *This research was supported by the National Institutes of Health NIH IRP. Contributions of NIH authors are considered Works of the US Government. Findings/conclusions presented herein do not necessarily reflect views of the U.S. NIH or HHS.

Th169. T Cell–dominated Interferon Signaling Drives Immune Checkpoint Inhibitor–associated Pneumonitis Revealing Actionable Cytokine Targets

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Abstract Text: Immune checkpoint inhibitors (ICI) have transformed cancer therapy by enhancing antitumor immunity but are frequently complicated by immune-related adverse events (IrAEs), including potentially life-threatening ICI-associated pneumonitis, for which the underlying immunopathogenesis remains poorly understood. To delineate the cellular and molecular drivers of lung IrAEs, we performed single-nuclei RNA sequencing on lung tissue biopsies from patients with ICI pneumonitis, pulmonary graft-versus-host disease (GvHD), connective tissue disease–associated interstitial lung disease (CTD-ILD), and healthy controls, followed by differential gene expression and pathway enrichment analyses. ICI pneumonitis was distinguished by a pronounced predominance of cytotoxic effector $CD8^+$ T cells, in contrast to CTD-ILD and pulmonary GvHD, which were enriched with tissue-resident memory $CD4^+$ T cells. Additionally, there was a distinct expansion of inflammatory macrophages in ICI pneumonitis compared with neutrophil-predominant profiles in other inflammatory lung diseases. Transcriptomic

analysis revealed significant upregulation of CXCL9, CXCR3, CD8A, IL21R, GZMB, CD38, JAK3, and STAT1 in ICI pneumonitis, consistent with heightened T Cell recruitment, activation, and effector function. Gene set enrichment analyses demonstrated robust activation of interferon response pathways and IL-6–JAK/STAT3 signaling, with relative suppression of TNF-α and hypoxia-associated programs, findings that were conserved when comparing ICI pneumonitis with healthy lung tissue as well as other inflammatory lung disease states. Collectively, these data identify a $CD8^+$ T Cell–dominant, interferon-driven inflammatory program as a defining feature of ICI-associated pneumonitis and highlight clinically actionable cytokine signaling pathways, including IL-6 and JAK/STAT3, as potential therapeutic targets to mitigate pulmonary IrAEs while preserving anti-tumor immunity. Future directions include using spatial transcriptomics to further explore/validate our findings, the development of an ICI-pneumonitis mouse model, and planning for early-phase clinical evaluation of targetable cytokine pathways.

Th170. HLA-DRB1*04 Dominance in Heterozygous Dendritic Cells Enhanced by SKBR3 Cell Lysate Antigenic Challenge

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Abstract Text: HLA class II antigen presentation is a critical determinant of $CD4^+$ T Cell activation, which contributes to the orchestration of the immune response. Effective $CD4^+$ T Cell responses depend on both the diversity and abundance of peptides presented, highlighting the importance of understanding how HLA-II polymorphism and heterozygosity shape peptide repertoires. Our previous research demonstrated that HLA-DR heterozygosity markedly modulates the peptide repertoires presented by HLA-DR molecules. While HLA-DRB1 polymorphism is known to critically shape the diversity of antigen presentation, the extent to which heterozygosity influences allele-specific peptide repertoires remains incompletely understood. These observations prompted us to investigate how a dominant allele, such as DRB1*04, behaves in heterozygous contexts and how its dominance is affected by exogenous antigen exposure, with potential implications for $CD4^+$ T Cell responses. Here, we analyzed monocyte-derived dendritic cells (MoDCs) from DRB1*04^+ heterozygous donors, to characterize the HLA-DR immunopeptidome under basal conditions and after pulsing with SKBR3 breast cancer lysates. Across four heterozygous contexts, DRB1*04 consistently displayed higher binding affinity and peptide exclusivity than its co-expressed alleles, except when paired with another immunodominant allele (DRB1*01:01), which contributed similarly to the peptide repertoire. Following antigenic pulsing, focusing on peptides derived from SKBR3-enriched proteins revealed further increases in DRB1*04 binding strength and exclusivity. Notably, although the identity of SKBR3-enriched peptides was largely shared between pulsed and control conditions, their abundance was consistently higher under basal conditions, suggesting that antigenic pulsing alters the presentation of constitutive (basal) proteins. These findings demonstrate that (i) DRB1*04 immunodominance is generally maintained in heterozygous contexts, (ii) selective uptake and processing of exogenous tumor proteins further enhance DRB1*04-mediated presentation of tumor-derived peptides, and (iii) antigenic pulsing alters the abundance, rather than the identity, of basally presented peptides. This work provides new insights into how HLA-DRB1 polymorphism and heterozygosity shape the class II immunopeptidome under antigen-challenged conditions, which could inform the design of personalized antigen-directed therapies.

Th171. ImmTAC-secreting T Cells as a Novel Therapeutic Platform for Melanoma

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Abstract Text: ImmTACs (Immune-mobilising monoclonal T cell receptors against cancer) 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 elicit anti-tumour responses by targeting peptide-loaded HLA complexes presented by cancer cells. Nevertheless, ImmTAC therapy requires weekly intravenous administration, which results in drug diffusion, low intratumoral concentrations, and systemic toxicity, limiting its potential therapeutic efficacy. Based on our experience developing chimeric antigen receptor (CAR)-T cell therapies for haematological malignancies, we sought to genetically engineer tumour-infiltrating lymphocytes (TILs) to secrete ImmTACs (ImmTAC-TILs), aiming to achieve T cell homing back to the tumour and localised delivery of the bispecific molecule. Here, we report the initial preclinical development of our ImmTAC-TIL therapy. We first investigated the feasibility of genetically modifying peripheral blood T cells to secrete the ImmTACs Tebentafusp and IMDA-001 (Immune Mobilising Diversiform Agent), an enhanced modified version of Tebentafusp incorporating a proprietary anti-CD3ζ scFv. ImmTAC-T cells were generated via lentiviral transduction of peripheral blood T cells at a low multiplicity of infection. ImmTAC-T cells expressing IMDA-001 exhibited a robust expansion comparable to untransduced (UTD) and CAR-19 T cells, with no significant differences in T cell subsets (N, EM, CM and TEMRA) or exhaustion markers throughout expansion. However, Tebentafusp-secreting ImmTAC-T cells displayed heightened levels of PD-1 and CTLA-4 and failed to expand as controls. Notably, IMDA-001 ImmTAC-T cells demonstrated potent cytotoxicity against HLA-A2^{+} gp100280-288-pulsed NALM6 B cells, comparable to CAR-19 T cells. They also efficiently mediated lysis of gp100^{+} patient-derived melanoma cell lines, with real-time cytotoxicity assays showing activity equivalent to soluble Tebentafusp. Furthermore, ImmTAC secretion by transduced T cells enabled ImmTAC decoration of non-transduced T cells, triggering melanoma cell lysis by untransduced T cells in transwell assays. Altogether, our preliminary data indicate that ImmTAC-T cells can be generated and expanded *in vitro* similarly to anti-CD19 CAR-T cells, mediating cytotoxic activity through a direct and bystander effect. These findings validate the feasibility and functionality of ImmTAC-T cells and support the further development of ImmTAC-TILs.

Th172. Nuclear and Cell Cycle-dependent Localization of CMTM6, a PD-L1 Stabilizer, in Cervical Cancer Cell Lines

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Abstract Text: Cervical cancer (CC) remains a leading cause of cancer-related mortality among women worldwide. Tumor immune evasion plays a central role in disease progression and is largely mediated by immune checkpoints that suppress antitumor immune responses. A key mechanism involves the PD-1/PD-L1 axis, where persistent PD-L1 expression on tumor cells enables immunosuppression. PD-L1 stability at the plasma membrane is regulated by CKLF-like MARVEL transmembrane domain-containing 6 (CMTM6), whose regulation and additional functions remain unclear. This study aimed to characterize the subcellular distribution and cell cycle dynamics of CMTM6 in cervical cancer cells and predict its interaction with PD-L1. To address this, cervical cancer cell lines were analyzed using immunofluorescence and confocal microscopy. Subsequent analyses combined cell cycle synchronization, subcellular fractionation and Western blotting to evaluate subcellular distribution dynamics. In parallel, an in silico structure-based approach was employed to predict the CMTM6-PD-L1 interaction interface using molecular docking analyses. Molecular docking identified a conserved PD-L1 interaction site at amino acid residue 228, supporting a stable CMTM6—PD-L1 interface. Additionally, the chaperone protein HSC70 was found to interact with PD-L1 within

the 171-265 amino acid region, consistent with previous reports and supporting competitive binding with CMTM6. Confocal microscopy revealed that CMTM6 localizes not only to the plasma membrane and cytoplasm but also exhibits a distinct and previously unreported nuclear localization. This nuclear pattern was not uniformly observed across all cells, suggesting regulated and phase-dependent nuclear translocation. Analyses of synchronized cells increased nuclear localization of CMTM6 during early S phase, followed by redistribution to the cytoplasm at later stages. Three-dimensional confocal reconstructions corroborated this observation, confirming precise intranuclear localization during defined cell cycle intervals. Collectively, these findings identify CMTM6 as a dynamically regulated, multifunctional protein in cervical cancer cells. Beyond stabilizing PD-L1, its cell cycle-associated nuclear localization suggests broader roles in tumor cell regulation. Together, these results highlight CMTM6 as a molecular hub connecting immune modulation with tumor cell regulation, underscoring its potential as a novel target for precision immunotherapy.

Th173. Spatial Multi-omic Profiling Uncovers Immune-tumor Niches Underlying Heterogeneity and Therapeutic Resistance in Clear Cell Renal Cell Carcinoma

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Abstract Text: Clear cell renal cell carcinoma (ccRCC) exhibits profound biological heterogeneity driven by dynamic tumor immune microenvironment interactions. Single-modality molecular profiling often underrepresents this complexity. Spatial transcriptomics provides high-resolution maps of gene expression, yet functional insights depend on proteins that mediate signaling, cellular states and immune responses. Sequential integration of Imaging Mass Cytometry™ (IMC™) technology following spatial transcriptomics on the same tissue section introduces a complementary layer of information by quantifying protein abundance and post-translational markers while preserving spatial context. Unlike fluorescence-based multiplexing, IMC technology has a single-step staining process that is gentle on tissues previously imaged on transcriptomic systems. FFPE tumor sections from a Stage 3 ccRCC patient were profiled using the Xenium 5K spatial transcriptomic assay (10x Genomics), followed by hematoxylin and eosin (H&E) staining and then a 43-marker IMC immuno-oncology antibody panel. All images were coregistered to achieve single-cell resolution integration of RNA expression, histology and protein markers. Integrated analysis offered definition of spatially organized tumor-immune interactions. Functional subtyping revealed metabolically active tumor populations ($LDHA^+$, $GLUT1^+$), $TIM\text{-}3^+$ tumor subsets with angiogenic potential, and tertiary lymphoid structures (TLS) enriched in B cells, activated T cells and macrophages. Combined transcriptomic and proteomic mapping captured cell–cell proximities among immune cells (CD4, CD8, CD20, CD38, TCF1, CD11c), stromal compartments (αSMA, vimentin, fibronectin), and vascular elements (CD34, PLVAP) associated with tissue remodeling and immune dysfunction. Unsupervised multi-omic clustering on combined RNA/protein data delineated immune activation within TLS and tumor cell states linked to metastatic potential. In addition, classifier-based supervised cell phenotyping revealed cellular neighborhoods with distinct immune–tumor cell interactions. IMC imaging performed after spatial transcriptomics gives per-cell RNA and protein data and supports a pathologist-in-the-loop workflow, bridging morphological assessment with molecular precision and enabling a spatially resolved understanding of immune evasion and therapeutic resistance. This integrative multi-omic framework advances the discovery of spatially defined biomarkers, facilitates patient stratification, and informs rational combination therapies targeting both immunologic and tumor-intrinsic pathways. For Research Use Only. Not for use in diagnostic procedures

Th174. Focusing on FLT4 to Exploit Anti-leukemic Precision Medicine Through Integrative Immunomodulatory Tools Within the Marrow Microenvironment

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Abstract Text: Objectives: Despite significant advancements in new drug development for acute myeloid leukemia (AML), over 30% of patients still encounter difficulties with relapse or drug resistance, highlighting substantial challenges in the treatment of AML. Recent studies have indicated a significant correlation between AML and lymphangiogenesis within the leukemic microenvironment, which facilitates tumor escape and recurrence. Additionally, the major lymphangiogenic factor, vascular endothelial growth factor receptor 3 (VEGFR-3 or FLT4), is significantly overexpressed in AML patients; however, the underlying mechanisms remain poorly understood. This study aimed to investigate the immunomodulatory effects of anti-VEGFR-3 peptide, either alone or in combination with various AML drugs, using an immunocompetent mouse model of AML. Methods: A syngeneic AML model was generated by intravenous injection of C1498-luc cells into C57BL/6 mice. Mice received mono-, dual-, or triple-combination treatments with an anti-VEGFR-3 peptide, the CXCR4 inhibitor plerixafor, and cytarabine (Ara-C). Immune responses were analyzed in peripheral blood, bone marrow, spleen, and liver by profiling T cells, natural killer (NK) cells, myeloid-derived suppressor cells (MDSCs), and regulatory T (Treg) cells. Treatment efficacy was assessed using bioluminescence imaging (BLI) and survival analysis. Results: Triple-combination therapy significantly enhanced IFN-γ production in peripheral blood $CD8^+$ T cells and increased IFN-γ expression in $CD4^+$ T cells and NK cells in the spleen and liver, indicating robust immune activation. Expression of immune exhaustion markers CTLA-4 and TIGIT was significantly reduced in T cells and NK cells, particularly in the liver. BLI demonstrated a marked reduction in tumor burden in the triple-treatment group by day 14. Dual treatment with anti-VEGFR-3 peptide and plerixafor significantly prolonged median survival compared with vehicle-treated controls. Conclusions: The study provides preliminary evidence that lymphatic factor-modulating peptides play a pivotal role when used alongside standard AML therapies, enhancing immune responses within the AML microenvironment. Further stepwise experiments and comprehensive immunological analyses are warranted to validate these findings and explore their therapeutic potential by exploring the exact action mechanism of VEGFR-3 blockade.

Th175. Evaluation of the Biomarker Potential of the tetraspanin33 Expression in B-cell Lymphomas

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Abstract Text: Many molecules have been identified in the development of different types of cancer, which are involved in migration, invasion, extracellular vesicles production, stemness and drug resistance. Thus, many therapeutic targets have been proposed for cancer treatment, such as tetraspanins. These proteins are a group of transmembrane proteins involved in cellular processes like signal transduction, motility, migration, membrane fusion and phagocytosis. Tetraspanin 33 (TSPAN33) is the most recently reported tetraspanin which expression has been characterized in some types of lymphoma and lymphoma-derived cell lines. "Lymphoma" is a term that comprehends a heterogenic group of neoplasms derived from cells of the lymphoid lineage: B, T or NK cells, and can be classified in Hodgkin lymphomas (HL) or non-Hodgkin Lymphomas (NHL) which can bear up to 50 different entities, varying in cell phenotype, prognostic, treatment and tetraspanin expression. In order to determine the link between the expression of TSPAN33 and clinical data, immunohistochemistry was performed on HL and NHL tissues and therefore correlated with clinical criteria such as treatment response, metastasis and outcome. Here we discovered that the expression of TSPAN33 has a negative correlation with treatment response and a positive correlation with metastasis. In conclusion, the TSPAN33 expression is a biomarker that can be used to predict the lymphoma

progression and can be used as a therapeutic target. supported by PAPIIT-DGAPA-UNAM IN215424 and SECIHTI CBF-2025-I-3132.

Tu163. Preclinical Examination of Tumor-targeted CD28-bispecific Antibodies in Combination with PD-1 and CD3-bispecific Immunotherapy

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Abstract Text: T cell activation is initiated upon binding of the T Cell Receptor (TCR)/CD3 complex to peptide-major histocompatibility complexes (“signal 1”); this activation is then enhanced by engagement of a second “co-stimulatory” receptor, such as the CD28 receptor on T cells binding to its cognate ligand(s) on the target cell (“signal 2”). CD3-based bispecific antibodies act by replacing conventional signal 1, linking T cells to tumor cells by binding a tumor-specific antigen (TSA) with one arm of the bispecific, and bridging to TCR/CD3 with the other. TSAxCD3 bispecifics have demonstrated promising anti-tumor efficacy in cancer patients, however their activity remains to be optimized, particularly in solid tumors. Herein we introduce a class of bispecific antibodies that mimic signal 2, by bridging TSA to the co-stimulatory CD28 receptor on T cells. When combined with TSAxCD3 bispecifics, they enhance the artificial synapse between a T cell and its target cell, potentiate T cell activation, and markedly improve anti-tumor activity of CD3-bispecifics in a variety of prostate and ovarian xenogeneic and syngeneic tumor models. In addition, we tested the combination with monoclonal antibodies that block the PD-1 checkpoint receptor. PD-1 antibodies have revolutionized cancer immunotherapy. However, many tumor types remain unresponsive to anti-PD1 therapy, and even amongst responsive tumor types, the majority of patients do not develop durable anti-tumor immunity. Herein we demonstrate that TSAxCD28 bispecifics (one specific for prostate cancer, and the other for epithelial tumors) can also synergize with the broader anti-PD-1 approach and endow responsiveness - as well as long-term immune memory - against tumors that otherwise do not respond to anti-PD1 alone. Unlike CD28 super-agonists, which broadly activate T cells and induce cytokine storm, TSAxCD28 bispecifics display little or no toxicity when used alone or in combination with a PD-1 blocker in genetically humanized immunocompetent mouse models or in primates.

Tu164. Cancer-associated Fibroblast-derived Factors Reprogram Neutrophil Functional Responses in Cervical Cancer

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Abstract Text: BACKGROUND: The tumor microenvironment plays a central role in cervical cancer (CC) progression, yet the mechanisms governing stromal–immune crosstalk remain incompletely defined. Cancer-associated fibroblasts (CAFs) and neutrophils are among the most abundant cellular components within cervical tumors, and both increase with disease progression. However, whether CAF-derived factors directly reprogram neutrophil functions is unknown. METHODS: CAFs were isolated from premalignant cervical lesions and early- and late-stage CC biopsies; the expression of FAP, α-SMA, and Vimentin markers confirms their phenotype.

Supernatants from these CAFs were used to stimulate peripheral blood neutrophils from clinically healthy donors. Functional responses were assessed by measuring cytokine secretion, apoptosis, metabolic activity, reactive oxygen species (ROS) production, degranulation marker expression (CD66b, neutrophil elastase, MMP-9), and neutrophil extracellular trap (NET) formation. RESULTS: CAF-derived factors induced a predominantly pro-inflammatory neutrophil profile characterized by increased secretion of the pro-inflammatory cytokines IL-6, IL-8, GM-CSF, TNF-α, and IFN-γ. Neutrophil survival was significantly prolonged, along with reduced apoptosis, after the stimulation with CC-derived CAF supernatants. Metabolic activity and ROS production were enhanced, particularly in response to late-stage CAFs. In parallel, activation and proteolytic markers were upregulated, consistent with increased degranulation. NETosis was significantly elevated in neutrophils stimulated with early-stage CAF-derived factors compared to untreated neutrophils. CONCLUSION: Our findings indicate that CAFs from CC secrete soluble mediators that promote functional polarization of neutrophils toward a predominantly protumoral functional phenotype. These results highlight CAF–neutrophil crosstalk as a potential driver of immune remodeling in cervical cancer and suggest this axis as a novel immunomodulatory strategy.

Tu165. DNA Hypomethylating Agents Preserve T Cell Stemness and Potentiate the Efficacy of CD3-bispecific Antibodies

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Abstract Text: Bispecific antibodies targeting tumor-associated antigens and CD3 are promising novel therapeutic agents for both solid and hematologic cancers. CD3-bispecifics induce T cell cytotoxicity and cytokine release; however, prolonged TCR stimulation can lead to chromatin rewiring and T cell dysfunction, thereby limiting their full therapeutic potential. We investigated the combination of CD3-bispecifics with the DNA hypomethylating agent decitabine and observed synergistic tumor growth inhibition in various preclinical models. Utilizing the PSMAxCD3 bispecific antibody for treatment of prostate carcinoma and *in vivo* humanized models, we cataloged, at the single-cell level, the dynamics of T cell epigenetic states during bispecific therapy and in combination with decitabine. Importantly, this combination strategy preserved a TCF-1^{+} T cell population and delayed acquisition of a dysfunctional state at both chromatin and protein levels. At the DNA methylation level, TCR stimulation in the presence of decitabine maintained a naive-like pattern in gene loci associated with T cell stemness. This study provides a rich resource for understanding the evolution of T cell states during immunotherapy and mechanistic support for combining epigenetic modifiers with CD3-bispecifics in the clinic.

Tu166. Clinical and Translational Validation of CD47-SIRPα Engineering Enables Context-Dependent Immune Circuit Reprogramming Across Oncology and Autoimmunity

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Abstract Text: Background CD47–SIRPα pathway is a myeloid immune checkpoint regulating macrophage phagocytosis and tissue immune hemostasis. While CD47 blockade has been explored in oncology, whether selective SIRPα-based engineering can enable controlled immune-circuit reprogramming across distinct disease

states remains unresolved. Methods We evaluated three engineered SIRPα-based fusion proteins designed to modulate innate immunity in complementary contexts. HCB101 (SIRPα) was evaluated in syngeneic tumor models and in an ongoing first-in-human Phase 1 dose-escalation study (NCT05892718) with Phase 1b/2a combination expansion (NCT06771622). HCB301 (SIRPα-PD-L1-TGFβ) was assessed in preclinical immune-signaling assays and in an ongoing Phase 1 study (NCT06487624). HCB206 (SIRPα-CD20) was evaluated in ADCP/ADCC/CDC assays and in an SLE patient-derived PBMC-engrafted humanized mouse model. Results Clinical and translational validation of innate-checkpoint modulation: Newly matured clinical data show that HCB101 achieved sustained CD47 receptor occupancy (≥90-99%) without reaching maximum-tolerated dose at 36 mg/kg and without cumulative anemia. Confirmed partial responses and durable disease stabilization were observed across tumor types. Combination cohorts maintained target engagement with manageable hematologic safety and no unexpected additive immune toxicity, supporting compatibility with standard-of-care targeted and checkpoint regimens. Translationally, HCB101 remodeled the tumor-immune microenvironment *in vivo*, increasing tumor-infiltrating leukocytes and macrophages, shifting polarization toward an M1-dominant phenotype (increased M1/M2 ratio), and enhancing $CD4^+$/$CD8^+$ T cells (including $CD69^+$ subsets) infiltration and activation. Multi-axis checkpoint integration: HCB301 integrates engineered SIRPα, anti-PD-L1, and an engineered TGFβ trapping into a single molecule, restoring T Cell activity, neutralizing TGFβ signaling, and enhancing macrophage-mediated phagocytosis in preclinical studies. Early clinical evaluations showed disease stabilization at Week 6, with predictable hematologic effects. Autoimmune translation with pathogenic selectivity: HCB206 demonstrated picomolar-range B-cell cytotoxicity and ~6-fold higher activity in primary SLE-patient B cells. In a SLE-PBMC-engrafted humanized model, HCB206 induced >90% depletion of splenic $CD19^+$ B-cells and achieved rapid suppression of serum anti-dsDNA IgG within 7 days, while showing negligible erythrocyte phagocytosis and minimal platelet uptake. Conclusions Three late-breaking clinical and translational findings establish selective SIRPα-based engineering of the CD47 axis as a programmable innate immune node capable of safe, context-dependent immune circuit reorganization across oncology and autoimmunity.

Tu167. Macrophage Hitchhiking Nanomedicine for Enhanced β-elemene Delivery and Tumor Therapy

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Abstract Text: Nanoparticle-based drug delivery systems hold promise for tumor therapy; however, they frequently encounter challenges such as low delivery efficiency and suboptimal efficacy. Engineered living cells can redirect drug delivery systems to effectively reach targeted sites. Here, we used living macrophages as vehicles, attaching them with GeS nanosheets (GeSNSs) carrying β-elemene for transport to tumor sites. GeSNSs act as efficient sonosensitizers, enhancing ultrasound-induced reactive oxygen species generation for treating 4T1 breast tumors. Notably, macrophage hitchhiking delivery of β-elemene–loaded GeSNSs not only achieves high accumulation in tumor regions and suppresses tumor growth under ultrasound treatment, but also effectively remodels the immunosuppressive tumor microenvironment by improving M1-like macrophage polarization and enhancing the populations of mature dendritic cells, $CD4^+$, and $CD8^+$ lymphocytes, thereby facilitating enhanced sonodynamic chemoimmunotherapy. These findings underscore the potential of macrophage hitchhiking strategy for drug delivery and suggest broader applicability of engineered living materials–mediated delivery technologies in disease therapy.

Tu168. IL-18 Modulates Regulatory T Cell Activity to Favour Anti-tumour Immunity in Melanoma

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Abstract Text: Immune checkpoint blockade has transformed cancer treatment, yet 33-50% of patients still exhibit poor responses. Treatment resistance is driven, in part, by regulatory T (Treg) cells through their potent ability to suppress anti-tumour immunity. However, they are essential for maintaining immune homeostasis and tolerance, making depletion infeasible due to off-target effects. Alarmins released upon tissue injury modulate T cell function. Among these, IL-18, a member of the IL-1 family, drives type-1 immunity. Previously, we demonstrated that IL-18 is essential for the Th1-like adaptation of Treg cells during acute and chronic infection. Here, we hypothesize that IL18R signalling modulates Treg cells to promote αPD-1 efficacy. Foxp3-RFP reporter mice bearing highly immunogenic YUMMER 1.7 melanoma tumours were treated with αPD-1 and stratified into high- and low-responders for flow cytometry and transcriptomic analysis (GeoMx®). High IL18R expression on Treg cells was a hallmark of high-responder mice and correlated with local enrichment of T cell activation, differentiation, and $CD8^+$ cytotoxicity signatures in situ. Further immunofluorescence studies confirmed that wild-type mice have a significantly greater proportion of Treg cells in the immunogenic tumour periphery relative to the core, a pattern absent in T cell-deficient IL18R ($CD4CreIL18R^{-/-}$) mice. Importantly, we also show that IL18R signalling is required for the *in vitro* adaptation of Treg cells into potent $Tbet^+IFN\gamma^+$ Th1-like cells and for αPD-1 efficacy *in vivo*. IL-18 also alleviated $IFN\gamma^+$ $CD8^+$ T cells from Treg-mediated suppression, further potentiating a strong type 1 inflammatory response. Additionally, tumour-bearing Treg-specific IL18R-flox ($Foxp3IL18R^{-/-}$) mice were analyzed via flow cytometry to determine phenotypic changes at the clinical endpoint. Preliminary results indicate that $Foxp3IL18R^{-/-}$ cells exhibit higher levels of CD25 and Helios, and lower levels of PD-1, Ki67, Foxp3, and CXCR3, highlighting a reduced activation state. This suggests increased proliferative and honing potential during active IL18R signalling. Collectively, these findings identify IL18 as a central regulator of Treg adaptation and spatial distribution during αPD-1 checkpoint blockade. Reprogramming Treg function through the IL-18R pathway represents a promising therapeutic strategy to reduce treatment resistance and enhance αPD-1 efficacy.

Tu169. High-throughput Peptide: MHC Screening of Recurrent Cancer Alterations and TAAs Reveals Shared Targets for Cancer Vaccines and TCR-T

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Abstract Text: Objectives: Cancer vaccine and TCR-T development are constrained by incomplete knowledge of which tumor-derived peptides are actually displayed by MHC-I. Existing discovery pipelines rely heavily on untargeted immunopeptidomics and prediction models trained on mass spectrometry datasets, both of which incompletely capture the desired cancer peptide space and often miss peptides containing reactive amino acids, such as cysteine. This limitation is particularly important in low tumor mutation burden cancers, where private neoantigen discovery is sparse. We applied EpiScan, a high-throughput functional genetics platform for pooled testing of predetermined peptides in mammalian cells, to experimentally nominate shared therapeutic targets from recurrent cancer mutations and tumor-associated antigens (TAAs). Methods: We used EpiScan to profile two cancer-focused peptide libraries across common U.S. HLA-I alleles. The first comprised approximately 15,000 peptides spanning approximately 350 recurrent cancer mutations. The second comprised approximately 53,000 peptides derived from about 50 TAAs. In EpiScan, peptide-dependent stabilization of cell-surface MHC-I enables pooled enrichment via FACS and sequencing-based recovery of displayed ligands. Results: EpiScan identified thousands of cancer-relevant peptide-MHC-I complexes from both recurrent mutations and TAAs across common alleles. Mutation-derived binders included peptides in which the altered residue created favorable anchor residues as well as peptides in which the mutation occupied more central, TCR-facing positions, consistent with multiple routes to neoepitope formation. Importantly, approximately 25% of identified ligands contained cysteine, representing greater than 10-fold enrichment over the expected recovery of cysteine-containing peptides by MHC

immunoprecipitation mass spectrometry. TAA screening markedly expanded the candidate target repertoire beyond mutation-only discovery, increasing target depth for tumors with low mutational burden, including prostate cancer. Conclusions: EpiScan enables high-throughput experimental access to a clinically actionable cancer epitope space that is incompletely captured by standard immunopeptidomics. By nominating shared, allele-resolved targets from both recurrent mutations and TAAs, this approach may support off-the-shelf and personalized cancer vaccines, improve target selection for low-TMB patients, and accelerate development of TCR-T therapies.

Tu170. Chemotherapy and EBV-specific T Cell Adoptive Immunity Modulate the Immunomes of Nasopharyngeal Carcinoma Patients

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Abstract Text: The systemic immunological mechanisms of adoptive T cell immunotherapy in nasopharyngeal carcinoma (NPC) are incompletely understood. Using samples from a multi-center, international randomised phase III trial, VANCE, we investigated remodeling of the immunome in patients with recurrent/metastatic (R/M) NPC after chemotherapy and subsequent immunotherapy with EBV-specific cytotoxic lymphocytes (EBV-CTLs) using high-dimensional mass cytometry. Baseline immune irregularities in untreated NPC compared to healthy controls showed significantly reduced naive T cells and total B cells, and elevated monocytes, NK cells, and T cells bearing the senescence marker CD57. Carboplatin/gemcitabine chemotherapy boosted some T cell populations, mostly expressing CD57 or immune checkpoint markers, as well as NK cells expressing CD62L and CD244. Subsequent infusion of EBV-CTLs modestly rescued the depletion of naive and transitional B cells in NPC, but frequencies of exhausted memory CD4 T cells as well as $CD244^+$ NK cells remained elevated. Senescent and terminally differentiated CD8 T cells also remained accumulated post chemotherapy and immunotherapy, suggesting that these therapies are insufficient for overcoming immune dysregulation in NPC, and that cells other than conventional T cells may play a role. In concordance with this idea, patients with above median overall survival (OS) had higher frequencies of unconventional $CD8^+$ MAIT and γδ T cells, as well as B cells, suggesting that these cells may compensate for defective anti-tumour T cell responses. Boosting the numbers or function of these cell types may be an effective avenue for enhancing anti-cancer immunity in NPC.

Tu171. Feasibility and Safety of Leukapheresis and CAR-T Cell Manufacturing in Low-weight Pediatric Patients with Relapsed/Refractory Acute Lymphoblastic Leukemia (R/R B-ALL)

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Abstract Text: Objectives Chimeric antigen receptor (CAR) T Cell therapy has improved outcomes in pediatric patients with R/R B-ALL. However, successful manufacture depends on adequate leukapheresis, which remains a challenge in very young and low-weight children. We report the outcomes of leukapheresis in a pediatric cohort weighing < 25kg. Methods Leukapheresis data from pediatric patients with r/r B-ALL weighing < 25 kg and eligible for CART19 therapy (tisagenlecleucel or ARI-0001) between January-2016 and December-2024 were retrospectively analyzed. All procedures were performed using the Spectra Optia system with donor blood priming and citrate–heparin anticoagulation. Results Twenty-two patients underwent 48 leukapheresis procedures, with only one repeat due to a manufacturing issue. Median age and weight at apheresis were 5.8 years (range 2–7) and 20 kg (range 10–25), respectively, and 62% were male. Patients had received a median of 2 prior therapies (range 2–5),

and 21 had previously undergone hematopoietic stem cell transplantation. Median pre-apheresis leukocyte count was 4×10^9/L (range 6.00–85.700), and median $CD3^+$ count was 1.35×10^9/L (range 2.16–6.474); 19 patients had circulating peripheral blood blasts (median 62%). Median blood volume was 1.390 mL (range 756–2.239), with a median of 3.5 blood volumes processed (range 2.3–5). All procedures were performed via central venous access, none in the intensive care unit, with a mean duration of 209 minutes (range 137–268). Intravenous calcium supplementation was routinely administered, and no citrate-related hypocalcaemia, hemorrhagic or infectious complications, or procedure-related mortality were observed. Six patients required a second leukapheresis to achieve the minimum $CD3^+$ cell threshold. Overall, 39 of 42 leukapheresis products (92.8%) were successfully manufactured without microbial contamination; five products were not infused due to disease progression, and during follow-up, 20 patients died, primarily from leukemia progression. Conclusions Leukapheresis and CAR-T cell manufacturing in pediatric patients with r/r B-ALL < 25 kg, including infants (< 1 year) and underweight patients, are feasible and safe, allowing sufficient T cells to be obtained in most patients in a single session without adverse effects. Increased institutional experience has been associated with improved manufacturing success rates.

Tu172. GPER Activation Inhibits Migration and Invasion in PCa Cell Lines: Functional and Transcriptomic Analysis

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Abstract Text: BACKGROUND: Prostate cancer (PCa) is the second most frequent disease worldwide, according to the World Health Organization (WHO). One of the biggest treatment challenges is controlling metastasis. G-protein-Coupled Estrogen Receptor (GPER), which has been linked to migration and invasion processes, has gained relevance in PCa development; however, there is no evidence of the molecular mechanisms involved in cell migration and invasion by GPER in PCa. To better understand these processes, we analyzed migration and invasion associated with differentially expressed genes (DEGs) to determine GPER's molecular regulation in PC3 and LNCaP cells. METHODS: PC3 and LNCaP cells were passaged until reaching 80% confluence in a 5% CO2 atmosphere at 37°C. PCa cells were stimulated with G1 (1 µg/ml) for 30 minutes, with three replicates. Subsequently, migration and invasion assays were performed using Transwell plates with 3x10^5 and 1x10^5 cells per well of PC3 and LNCaP, respectively, for 24 and 48 hours. Additionally, RNA was isolated from PC3 and LNCaP cells after G1 stimulation (1 µg/ml) for bulk RNA sequencing (RNA-Seq) by Novogene Corporation Inc.; data were processed and analyzed using the Galaxy platform and the iDEP web application. RESULTS: RNA-seq analysis identified 15 upregulated and 8,235 downregulated genes in both PC3 and LNCaP G1-stimulated versus basal conditions. The most significantly (FDR< 0.05, fold-change>2) upregulated genes include ANGPTL4, IL13RA2, and PLIN2 in PC3 cells, and EGR1, FOS, and CBLN2 in LNCaP cells. Downregulated genes include UBA7, IFITM1, and NLRC5 in PC3 cells, KLK3, TMPRSS2, and NKX3-1 in LNCaP cells. GO enrichment analysis demonstrated significant downregulation of estrogen metabolic process related genes and pathways in LNCaP cells upon GPER activation; further research is needed. CONCLUSION: GPER activation decreases migration and invasion and influences molecular processes in PCa cell lines, offering new insights into the antitumoral role of GPER and its significance in cancer progression.
Funding: CONAHCYT project CBF2023-2024-2702, Basic and Frontier Science 2023–2024.

W164. Intratumoral Biodegradable-nanofluidic Platform for Localized Multimodal Immunotherapy Enhances Tumor Eradication and Immune Memory

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Abstract Text: Tumor-infiltrating lymphocytes are essential in shaping the tumor immune microenvironment, with higher levels often correlating with better outcomes. Although immunotherapy has transformative potential, its success is frequently limited by resistance—especially when using single or dual agents—and by systemic toxicity from multi-drug regimens. To overcome this, we developed a biodegradable nanofluidic drug-eluting seed (b-NDES) platform for sustained, localized release of multiple immunotherapeutics. We loaded these implants with immune checkpoint inhibitors (α-CTLA4), immune adjuvants (STING agonist, resiquimod), IL-12, and agonist antibodies (α-CD40), delivering them intratumorally in mouse models of 4T1 triple-negative breast cancer (TNBC), KPC pancreatic cancer, and KLN205 lung cancer. In the 4T1 model, we tested three-, four-, and five-drug combinations to evaluate tumor eradication. The b-NDES platform markedly improved control, with the five-drug regimen most effective—achieving complete clearance in three of six mice. This effect was consistent across KPC and KLN205 models, demonstrating robust efficacy in diverse tumors. Rechallenge experiments in 4T1 further revealed durable immune memory, with mice rejecting subsequent inoculation and T cells recognizing tumor cells via IFN-γ secretion. In KPC abscopal models, the five-drug regimen induced systemic immune activation, driving regression of untreated distant tumors. Serum cytokine and chemokine profiling via Olink identified a strong pro-inflammatory signature correlating with enhanced anti-tumor immunity. Compared to systemic intraperitoneal injections, which triggered cytokine storms, b-NDES delivery minimized toxicity, maintaining stable body weight and temperature with fewer liver changes. This b-NDES platform offers an innovative, localized approach to multimodal immunotherapy, achieving potent eradication, systemic immunity, and long-term memory with reduced toxicity. These findings underscore its potential to advance safer, more effective treatments for aggressive cancers.

W165. Translational Application of TEAD Inhibitors to Overcome Immunotherapy Resistance in Solid Tumors: Clinical and Preclinical Mechanistic Supportive Data

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Abstract Text: The role of tumor-associated macrophages (TAMs) compromising the efficacy of immune checkpoint inhibitor (ICI) agents as well as CAR T Cell therapy is described in solid tumors. Our investigation of tumor immune microenvironment (TIME) in malignant pleural mesothelioma (MPM, N=267) showed 2-10% tumor-infiltrating immune cells are $PD1^+$ T cells ($CD8^+$ and $CD4^+$); however, a 1:1 ratio of $PD1^+$ T cells to TAMs is observed underscoring their close interaction in shaping antitumor immunity. Our ongoing clinical investigation in MPM tumors demonstrated that loss of the tumor suppressor Neurofibromatosis Type 2 (NF2), a key regulator of the Hippo pathway, is associated with resistance to ICI immunotherapy in MPM. In both immunocompetent and immunodeficient preclinical models we observed that NF2 loss induces constitutive YAP/TAZ activation and non-cell-autonomously remodels the TIME, resulting in increased chemokine secretion and accumulation of immunosuppressive TAMs, which in turn directly mediate resistance to both anti-PD-1 and CAR T Cell therapies. TEAD inhibitors (TEADi), which block YAP/TAZ transcriptional activity downstream of Hippo signaling, are under evaluation in preclinical and clinical studies in solid tumors for tumor-intrinsic effects. Here, we investigated the immunological effects of TEAD inhibition in NF2-deficient MPM preclinical models using mesothelin-targeted CAR T cells (M28z), currently in phase I/II clinical studies from our lab. Combination therapy with TEADi and low-dose CAR T cells significantly improved tumor control and survival (tumor control up to day 50 vs. day 15 with CAR T cells alone in NF2-altered tumors). This therapeutic benefit correlated with reduced TAMs infiltration assessed by F4/80,

CD206, CRIg markers, and enhanced CAR T Cell proliferation and effector function. Our observations reveal a previously unrecognized NF2-Hippo-macrophage axis driving immunotherapy resistance. Furthermore, our investigations support a previously undescribed mechanistic rationale to use TEADi as a therapeutic strategy to remodel the TIME and provide a mechanistic framework for restoring CAR T Cell sensitivity in solid tumors harboring NF2 alterations.

W166. Viral Reactivation Following CAR-T Cell Therapy in Pediatric Patients: A Single Tertiary Center Experience

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Abstract Text: Objectives Patients receiving chimeric antigen receptor (CAR) T Cell therapy are at high risk of infections (incidence of 17-56% in adults); data on viral reactivation in pediatric populations are limited. This study aimed to evaluate the incidence, clinical characteristics, and prognostic impact of viral reactivation in pediatric patients after CAR-T therapy. Methods We conducted a retrospective study of pediatric patients with B-cell acute lymphoblastic leukemia (B-ALL) undergoing CAR-T between 2016-2025 who had any subsequent viral reactivation (presence of two determinations above the technique's quantification limit). Cytomegalovirus (CMV), Epstein–Barr virus (EBV), and adenovirus (ADV) PCR testing was performed prior to lymphodepletion chemotherapy (LC) and weekly during the first month post-CAR-T. The follow-up period was from LC until loss of CAR-T, treatment with haematopoietic stem cell transplantation (HSCT), loss to follow-up or death. Results A total of 102 patients received CAR-T-CD19 therapy (Tisagenlecleucel=79; ARI-0001=23), seven (6%) experienced viral reactivation; median age at diagnosis of 3.4 years (range, 0.2–21). Patients had received a median of three prior treatment lines (range, 2–5), including two with previous HSCT. Three patients developed grade 3–4 CRS and two had ICANS≥grade 2. Corticosteroids were administered in three patients (median duration, 10 days). Four patients had isolated CMV reactivation, one EBV, one ADV, and one sequential CMV and ADV reactivation. Median peak viral load for CMV was 2,286copies/mL (range, 429–13,097) over a median of 14 days. EBV and ADV reactivations peaked at 6,278 and 397copies/mL, at 18 and 7 days respectively. Median lymphocyte count prior to lymphodepletion was 400/mm³ (range 300-2.100), and median CD4 count at reactivation was 100/mm³ (range, 0-587). No patients received antiviral prophylaxis. Antiviral treatment was required in five patients (3 cidofovir, 2 foscarnet), with good clinical response. Only one patient developed symptoms (diarrhea associated with ADV). Four patients relapsed and died due to disease progression. Conclusions In this cohort of over 100 pediatric B-ALL patients treated with CAR-T therapy, viral reactivation incidence was low (6%) and mostly asymptomatic. This may be related to routine immunoglobulin replacement therapy, administered in most patients with a target trough level ≥8 g/L. Antiviral treatment was effective when required.

W167. Longitudinal Tracking of TCR Clonotypes in FFPE Breast Cancer Samples Reveals Potential Biomarkers for Tumor Reactivity and irAEs

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Abstract Text: The activation of tumor-infiltrating lymphocytes (TILs) following immune checkpoint inhibitor (ICI) therapy remains unpredictable, largely due to limited understanding of the antigenic targets recognized by their T cell receptors (TCRs). While tumor-specific T cells can mediate effective anti-tumor responses, bystander or cross-reactive lymphocytes may also become activated, potentially recognizing self-antigens and causing immune-related

adverse events (irAEs). These toxicities are often detected late, as molecular immune monitoring is not routinely implemented, limiting early intervention and patient-specific management. Sequential FFPE samples were analyzed from a breast cancer patient treated with pembrolizumab (anti–PD-1) who developed immune-related pericarditis (irAE) approximately one year after treatment. Samples included two diagnostic tumor biopsies, two on-treatment tumor biopsies, a surgical tumor specimen with associated lymph nodes, and a pericardium biopsy collected at the time of irAE. Genomic DNA was extracted from FFPE sections, and TCRβ repertoires were amplified using the DriverMap™ AIR TCR-BCR Profiling Kit (Human DNA). Libraries were sequenced and analyzed with Platforma Bio (MiLaboratories), allowing high-resolution clonotype identification. Using this DNA-based NGS workflow, we confirmed the presence of clonotypes previously detected at the RNA level in the group's earlier experiments. The clonotype CAISPSSIDRFGYTF was detected in all tumor samples and the surgical lymph node. CASSELGDTQYF was detected in all tumor samples and the pericardium. Additionally, CASSSDPGTYEQYF was present in all tumor and pericardial samples and has been previously identified in peripheral blood of a Type 1 diabetic patient, suggesting potential cross-reactivity or bystander expansion. These results demonstrate that the DNA-based NGS approach enables robust and reproducible longitudinal tracking of dominant T cell clones across tumors and inflamed tissues affected by irAEs. This study highlights the feasibility of FFPE-derived TCRβ profiling for retrospective and prospective immune monitoring. Persistent and shared clonotypes across tumor and irAE-affected tissues may serve as biomarkers for tumor-reactive T cells and early detection of immune-related toxicity, providing a framework for patient-specific monitoring during ICI therapy.

W168. Immune Checkpoint Expression on $CD8^+CD28^+$ and $CD8^+CD28^-$ T Cells in Cervical Cancer: Insights into Exhaustion and Senescence

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Abstract Text: Cervical cancer remains a leading cause of death among women worldwide, particularly in developing countries such as Mexico, where it is the second leading cause of cancer-related mortality. $CD8^+$ T cells are essential for controlling disease progression; however, their fate is shaped by the tumor microenvironment, which promotes exhaustion and/or senescence, two major dysfunctional states in cancer. Loss of CD28, a key costimulatory receptor, may provide valuable insight into the development of these dysfunctional T cell states. In addition, while the co-expression of multiple immune checkpoints is a well-recognized feature of T cell exhaustion, its presence and relevance in senescence remain poorly understood. The aim of this study was to characterize immune checkpoint expression on $CD8^+$ T cells according to the presence or absence of CD28 from patients with cervical cancer. In this ongoing study, patients with cervical cancer and healthy donors are being enrolled, and immune checkpoint expression on $CD8^+$ T cells according to CD28 expression status is being analyzed by multiparametric flow cytometry. Results to date show an increased frequency of $CD8^+CD28^-$ T cells compared with healthy donors. Within this subset, several immune checkpoints, including PD-1, NKG2A, and TIM-3, display lower expression levels, whereas TIGIT is upregulated. In contrast, $CD8^+CD28^+$ T cells exhibit increased expression of PD-1, TIGIT, NKG2A, and TIM-3 compared with healthy donors. Together, these results underscore the heterogeneity of $CD8^+$ T cell dysfunction in cervical cancer and suggest that CD28 status may be relevant for understanding immune checkpoint–based therapeutic strategies.

W169. Microsatellite Instability in Prostate Cancer: NGS vs Immunohistochemistry Methods and Its Association with Tumor-infiltrating Lymphocytes and PD-1/PD-L1 Expression

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Abstract Text: BACKGROUND: Prostate cancer (PCa) is among the most common cancers in men globally. Although PCa typically exhibits an immunosuppressive microenvironment with low tumor-infiltrating lymphocytes (TILs), patients with Microsatellite instability-high/Deficient Mismatch Repair (MSI-H/dMMR) may benefit from immune checkpoint inhibitors (ICIs). In clinical practice, MSI is usually assessed by immunohistochemistry (IHC) of MMR proteins, however, Next Generation Sequencing (NGS) offers more accurate results. This exploratory study compares MSI detection by NGS vs IHC and identifies the immune microenvironment through $CD4^+/CD8^+$ lymphocyte ratios and PD-1/PD-L1 expression in tumor tissues from Mexican PCa patients. METHODS: Seven formalin-fixed paraffin-embedded tumor biopsies from Mexican PCa patients were collected. The Gleason score was assessed, and MSI status was determined using NGS with five markers (NR-27, NR-21, NR-24, BAT-25, BAT-26) and IHC for MLH1, MSH2, MSH6, and PMS2 proteins. TILs were analyzed using CD3, CD4, and CD8 markers, and PD-1, PD-L1 expression was evaluated in tumor cells, immune cells, and endothelium. RESULTS: NGS identified 71.4% cases as Microsatellite Stability (MSS) and 28.6% (n=2) as Microsatellite Instability-Low (MSI-L). IHC was performed in 3/7 cases; two (classified as MSI-L by NGS) showed a low percentage of cancer cells stained with MSH2/MSH6 resulting in discrepancy in 2 cases reported as MSI-L by NGS. All cases displayed a mixed, low (5-25%) lymphocyte distribution, except for one with a moderate (26-50%) distribution and exhibited a higher ratio of $CD8^+$ cells. PD-L1 staining was negative in tumor cells. In immune cells, 57%(n=4) of cases showed a lower (1-5%) tumor area occupied by PD-L1 positive immune cells, and 14.3%(n=1) of cases showed a moderate (6-10%) tumor area with PD-L1 staining immune cells. PD-L1 expression in endothelial cells was observed in 28.6% of cases. CONCLUSION: This preliminary finding suggests discrepancy between NGS and IHC MSI assessment in PCa. CD8 predominance in the immune microenvironment with no PD-L1 expression in tumor cells was observed. Moreover, PD-L1 expression in endothelial cells suggests potential vascular-mediated immune evasion mechanisms. Larger studies are necessary to validate these preliminary findings and explore implications for patient selection for ICIs in PCa. Funding: COECyTJAL, 2025 Fondo de Desarrollo Científico de Jalisco (FODECIJAL) para Atender Retos Sociales, Folio 11918

W171. Type I Interferon Signaling Instills Divergent Metastatic Phenotypes and Immunotherapy Responses

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Abstract Text: Metastatic colonization of distant tissues is responsible for most cancer deaths, yet while lymph node (LN) metastasis typically precedes distant metastasis, the clonal and functional relationships linking these metastases remain unclear. Reconstruction of metastatic phylogenies in mouse models and patients suggests that LN and distant organ metastases are often seeded by independent clones, yet no genetic drivers have bene found to account for this divergent seeding. Here, we discover opposing roles for interferon signaling in metastasis to LNs and distant organs. We uncover that exposure to leukocyte-derived type I interferon promotes LN metastasis while impairing spread to distant organs by inducing interferon-stimulated gene (ISG) expression. Furthermore, while LN metastases have previously been demonstrated to disrupt anti-tumor immunity, we find that ISG expression induced during LN metastasis sensitizes tumors to immune checkpoint blockade (ICB). By mimicking the phenotype of LN metastases through delivery of type I interferon, ICB refractory primary tumors and distant metastases can be sensitized to ICB. Collectively, these findings demonstrate that the immune system conditions tumors for LN

metastasis through type I interferon secretion while impairing distant spread and that this axis can be exploited to render immunotherapy resistant tumors sensitive to immune checkpoint blockade therapy.

Infectious Diseases

Th176. Exosome-assisted Electrochemical Genosensing for IFNγ mRNA Detection in Immune Monitoring

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Abstract Text: Interferon-gamma (IFNγ) is a key cytokine orchestrating immune responses in contexts such as infections, cancer, and autoimmune diseases. Its role as a dynamic biomarker in immune regulation makes its accurate detection essential for clinical and research applications. Conventional detection techniques (ELISA, RT-PCR) are robust but often lack the capacity for early detection and minimally invasive sampling. This approach was used to evaluate the cellular immune response in SARs-CoV-2 infeccted and previously vaccinated individuals after incubating blood samples directly with spike and nucleocapside peptide mixture. In this work, we present an exosome-based electrochemical genosensing approach for the sensitive detection of IFNγ mRNA, offering a promising tool for real-time immune monitoring.The method leverages immunomagnetic isolation of exosomes derived from peripheral blood mononuclear cells (PBMCs), using anti-HLA-DR functionalized particles to selectively capture MHC class II-positive vesicles secreted by activated immune cells. This strategy circumvents the need for ultracentrifugation, enabling streamlined sample processing. Captured exosomes undergo molecular profiling via an electrochemical genosensor that integrates reverse transcription with a double-tagged endpoint PCR amplification. The system targets both IFNγ and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as a housekeeping control. Compared to bulk extracellular RNA detection, the use of HLA-DR$^+$ exosome enrichment significantly enhances sensitivity and specificity for IFNγ transcript detection. The platform provides a miniaturized and sensitive alternative to conventional assays for the detection of IFNγ mRNA that can be used directly in plasma samples. The exosome-based genosensing approach enables nucleic acid-based monitoring of immune-related biomarkers in isolated extracellular vesicles, with potential applicability in clinical contexts such as oncology, infectious diseases, and autoimmune conditions.

Th177. Immunomics Identifies Biomarkers of Post-Covid Syndrome ME/CFS and of Response to Immunoadsorption Therapy

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Abstract Text: COVID-19 infections can lead to symptoms of chronic fatigue and exertion intolerance, persisting for months and even years post-infection - known as Post-Covid Syndrome (PCS) or Long-Covid. The estimated incidence is about 400.000 new cases in Germany every year and no prescribable therapies exist. The heterogeneous clinical appearance points towards multiple etiologies and poses significant challenges in establishing diagnostic criteria. A subset of PCS patients develops myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), a severe postinfectious sequela of various infections. Clinical studies provided strong evidence for the role of autoantibodies in ME/CFS and show that about two thirds of patients benefit from immunoglobulin removal (immunoadsorption, IA). However, a detailed understanding of why some patients benefit from IA is lacking. To reveal immune alterations indicative of IA-response, cytometry by time of flight (CyTOF) was used to profile leukocytes in blood samples from PCS-ME/CFS patients pre- and post-treatment and age- and sex-matched controls. PCS-ME/CFS patients divided into IA-responders and non-responders. We observed an increased B cell activation in PCS-ME/CFS patients compared to controls. Importantly, this was exclusively evident in pre-treatment samples of IA-responders, potentially enabling patient stratification in future clinical trials ahead. Furthermore, dysregulations in the B cell compartment normalized at the end of treatment, suggesting a causal link. These results, critically, extended to multiple PCS-ME/CFS cohorts. Using plasma proteomics, we revealed a distinct inflammatory signature specific for a subgroup of patients, independent of B cell analyses. This study presents specific B cell alterations as potential biomarkers for PCS-ME/CFS and autoantibody-targeting treatment. Currently, we perform single-cell transcriptome and immunoreceptor sequencing, and mechanistic *in vitro* analyses to understand which factors drive these immune dysregulations and their contribution to PCS development. However, the study also highlights the complexity of PCS and the need to identify biomarkers predicting therapy response and further demonstrates that multimodal approaches might be necessary for future diagnostics.

Th178. Corticosteroids Drive Oral Candidiasis Through Impairing Type 17-immunity Without Causing Interferon-gammopathy

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Abstract Text: Candida albicans is a normal member of the human microbiome in the mouth and several other body sites, but it can also cause infections in susceptible individuals. These include oropharyngeal candidiasis (OPC), as well as other mucocutaneous and systemic infections. Oral infection susceptibility is often due to iatrogenic exposures – most commonly corticosteroids. Despite an extensive literature on murine OPC using the cortisone acetate model, susceptibility in this model has not been mechanistically interrogated. Here we show, using genetic models, that depression of type 17 immunity is the primary mechanism of susceptibility in cortisone-induced OPC. In contrast, interferon-gammopathy, another immune state that can lead to oral candidiasis, does not play a role in cortisone-induced OPC. We further investigated the interaction between type 17 immune deficiency and interferon-gammopathy, using both pharmacologic and genetic studies. We found that interferon-gammopathy is not needed for OPC susceptibility during type 17 deficiency. Thus, deficiency in type 17 immunity and interferon-gammopathy act independently of each other in the pathogenesis of oropharyngeal candidiasis.

Th179. The Price of Exhaustion: Mapping the Immune State in Patients with ACLF from Western Mexico

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Abstract Text: Liver cirrhosis is the sixth leading cause of mortality in Mexico, with chronic ethanol consumption as a primary etiological factor. In these patients, infections are the main triggers of clinical decompensation and Acute-on-Chronic Liver Failure (ACLF), a syndrome defined by intense systemic inflammation and multi-organ failure. A critical component of this progression is the development of an exhausted T cell phenotype, where the immune system fails to effectively clear infections due to the upregulation of inhibitory receptors. Given that Latino populations experience higher short-term mortality compared to European cohorts, understanding the role of T cell exhaustion in the context of acute decompensation is of clinical priority. The aim of this study is to characterize the expression of immune checkpoints on T lymphocytes from Mexican patients with decompensated cirrhosis without ACLF, with ACLF, and healthy controls, which has not been investigated Fresh whole blood samples from patients and healthy donors were analyzed by multiparametric flow cytometry. The results show an increased frequency of $CD4^+$ T cells expressing immune checkpoints in patients compared with healthy donors. Specifically, there is a higher frequency of $CD4^+$ T cells positive for markers such as PD-1 and BTLA in both the decompensated and ACLF groups. These findings underscore a pattern of T cell exhaustion within the Mexican population. This exhausted phenotype, particularly within the $CD4^+$ compartment could potentially be associated with an impaired ability to control infections, thereby contributing to the severe clinical course and high mortality rates observed in Latino patients with cirrhosis.

Tu173. Dynamics of EBA-175–specific Antibodies and Their Association with VAR2CSA Responses in Primigravid Women During the Postpartum Period

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Abstract Text: Background: Malaria in pregnancy (MiP) remains a major contributor to maternal and neonatal morbidity in sub-Saharan Africa. While immune responses during pregnancy have been extensively studied, the postpartum period remains a vulnerable and poorly characterized window. Erythrocyte-binding antigen 175 (EBA-175), a key merozoite ligand involved in erythrocyte invasion, is an important target of naturally acquired immunity, yet its postpartum dynamics in primigravid women are not well understood. This study assessed antibody responses to EBA-175 and explored their relationship with malaria infection status and VAR2CSA-specific immunity. Methods: Thirty-three primigravid women from Nanoro, Burkina Faso, were enrolled at delivery and followed at one and three months postpartum. IgG responses to EBA-175 region II were measured by ELISA, and serostatus to VAR2CSA (ID1-ID2a) IgG1 and IgG3 was used to evaluate potential co-acquisition of immune responses. Group comparisons were performed using Wilcoxon rank-sum and Fisher's exact tests. Results: Women seropositive for VAR2CSA-specific IgG1 exhibited higher mean EBA-175 antibody levels at delivery and one month postpartum, although differences were not statistically significant. IgG3 seropositivity to ID1-ID2a showed a stronger trend, with higher EBA-175 levels at one month postpartum (2.26 vs. 1.51 OD; $p = 0.072$). Across all groups, EBA-175 antibody levels declined markedly by three months postpartum. No association was observed between EBA-175 levels and peripheral parasitaemia at delivery. Conclusion: These findings suggest partial co-acquisition of immune responses to placental (VAR2CSA) and merozoite (EBA-175) antigens in primigravid women, with IgG3 showing the strongest temporal association. The rapid decline of EBA-175 antibodies postpartum highlights dynamic immunological changes during this period. Larger studies are needed to clarify the role of EBA-175–specific immunity in postpartum malaria susceptibility.

Tu174. HIF1α-driven Glycolytic Reprogramming Inhibits Mycobacterium tuberculosis Growth in Macrophages

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Abstract Text: Objectives: This study aims to elucidate how metabolic shifts, specifically the HIF1α-mediated glycolytic pathway, influence the ability of host to control Mycobacterium tuberculosis (Mtb) within mouse bone marrow-derived macrophages (BMDMs). Methods: We utilized RNA-sequencing to profile transcriptomic alterations in Mtb-infected BMDMs. The functional impact of glycolysis was evaluated by modulating glucose availability and pharmacological regulation of HIF1α using an agonist and the inhibitor FM19G11. Furthermore, the role of succinate as a metabolic signal for HIF1α stabilization was examined through exogenous treatment. Results: Mtb infection significantly induced glycolytic gene expression and HIF1α signaling. Enhanced glycolytic flux, driven by high glucose or HIF1α activation, boosted the production of reactive oxygen species (ROS) and proinflammatory cytokines, thereby limiting intracellular Mtb growth. Conversely, HIF1α inhibition facilitated bacterial persistence. Importantly, exogenous succinate treatment promoted HIF1α stabilization and augmented glycolytic flux, effectively restricting Mtb survival. Mechanistically, succinate was identified as a critical mediator that stabilizes HIF1α, promoting a proinflammatory M1-like phenotype and glycolytic metabolism. Conclusions: Our findings demonstrate that the succinate-HIF1α axis is a fundamental host defense mechanism. Inducing glycolytic reprogramming via this axis is essential for the effective restriction of Mtb survival in macrophages.

Tu175. Berberine Promotes Host Lipolysis to Enhance Antimicrobial Defense Against Hypervirulent Klebsiella Pneumoniae Infection

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Abstract Text: Hypervirulent Klebsiella pneumoniae (hvKp) is an emerging pathogen that causes severe community-acquired infections; however, the immune mechanisms controlling intracellular hvKp remain poorly understood. Here, we investigated the therapeutic potential of berberine against hvKp infection and elucidated its underlying molecular mechanisms using macrophage cell models and zebrafish *in vivo* models. We demonstrated that berberine significantly reduced intracellular hvKp survival in macrophages and improved survival rates in hvKp-infected zebrafish. Berberine markedly attenuated proinflammatory cytokine production by inhibiting the c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) signaling pathways. Notably, we found that hvKp exploited host lipid droplet (LD) biosynthesis for intracellular survival, and berberine effectively suppressed LD accumulation. Mechanistically, berberine promoted the nuclear translocation of transcription factor EB (TFEB), leading to enhanced lipolysis. Although berberine upregulated autophagy-related gene expression during hvKp infection, it did not induce lipophagy—the selective autophagic degradation of LDs. These findings collectively demonstrate that berberine serves as a promising therapeutic agent against hvKp infections by modulating host lipid metabolism to restrict bacterial intracellular survival.

Tu176. Broad Inhibition of Coronaviruses by an IgM Antibody Targeting a Conserved Spike Fusion Peptide

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Abstract Text: SARS-CoV-2 remains a major concern due to the ongoing emergence of variants that evade pre-existing neutralizing antibodies against the viral spike protein. Antibodies recognizing conserved regions within the spike could provide cross-variant protection; however, induction of IgG may increase the risk of antibody-dependent enhancement (ADE). We therefore aimed to develop an IgM antibody targeting the fusion peptide, a highly conserved region across coronaviruses, and to evaluate its antiviral efficacy *in vitro* and *in vivo*. From mice immunized with an amino acid sequence in the spike fusion peptide (SAIEDLLFNKV; CoV peptide), a monoclonal IgG antibody (anti-CoV IgG) specific for the fusion peptide core was generated and subsequently used to engineer a chimeric human IgM antibody (anti-CoV IgM).These antibodies bound the spike of SARS-CoV-2, SARS-CoV, and MERS-CoV in ELISA and Western blotting. Competitive ELISAs using the CoV peptide showed dose-dependent inhibition of antibody binding, with anti-CoV IgM exhibiting approximately 100-fold higher affinity than anti-CoV IgG. Anti-CoV IgM suppressed progeny virus production in cells infected with mouse hepatitis virus and with the Wuhan, Delta, and Omicron variants of SARS-CoV-2, whereas anti-CoV IgG did not at the same concentrations. All five human ACE2–transgenic mice receiving intravenous anti-CoV IgM survived to day 10 after lethal challenge with the Delta variant, whereas two of five mice treated with control IgM died. These findings demonstrate that anti-CoV IgM confers broad protection against coronavirus infections and may offer a promising strategy for combating current and future SARS-CoV-2 variants without ADE.

Tu177. Safety, Immunogenicity and Efficacy of a Human Lymphatic Filariasis Vaccine, LFGuardTM

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Abstract Text: Lymphatic filariasis is a neglected tropical disease that causes chronic disability and irreversible damage to the lymphatic system. Despite decades of mass drug administration campaigns aimed at interrupting transmission, millions of people in endemic regions remain infected. A prophylactic vaccine will be essential to achieving herd immunity and ensuring the long-term elimination and eventual eradication of the disease. Our laboratory developed a recombinant protein vaccine, rBmHAXT, which demonstrated strong immunogenicity and robust protection against challenge infections in both rodent and non-human primate models. A clinical-grade formulation of this vaccine, designated LFGuard™, has been manufactured in compliance with current Good Manufacturing Practice (cGMP) guidelines. In this study, we evaluated the safety, immunogenicity, and efficacy of the LFGuard™ vaccine in a mouse model. The Al-T4™ adjuvant, an alum-adsorbed TLR4 agonist produced by PAI Life Sciences, Inc. (Seattle, WA), known to promote a balanced Th1/Th2 response, was used to formulate the vaccine. Safety assessments including body weight monitoring, haematological analyses, and liver and kidney function tests revealed no adverse effects in any vaccinated groups compared to controls. LFGuard™ elicited a strong immune response, inducing high antigen-specific IgG titers (1:80,000). In challenge studies, LFGuard™ conferred significant protection, reducing infection by 90.7% relative to controls. These findings demonstrate that the cGMP-manufactured LFGuard™ vaccine is safe, immunogenic, and highly protective in the mouse model, supporting its advancement as the first human lymphatic filariasis vaccine to enter clinical trials.

W172. Utilizing the VSV Vector Approach for Emerging Infections Disease Vaccine Development

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Abstract Text: Better outbreak preparedness is a global priority. Using proven vaccine technology can accelerate vaccine development for outbreak pathogens. A highly effective Ebola virus (EBOV) vaccine marketed as ERVEBO® is based on the replication competent recombinant Vesicular Stomatitis Virus (rVSV) vaccine delivery vector. ERVEBO® was generated by replacing the VSV envelope glycoprotein with the Zaire EBOV glycoprotein (ZEBOV-GP), giving rise to VSVΔG-ZEBOV-GP. The rVSV∆G vector technology is versatile and can be applied to other viral pathogens; it is safe, provides durable immunity after single administration and induces protective immunity rapidly. ERVEBO® has already been approved by the European Commission, the US Food and Drug Administration and regulators in numerous countries. IAVI's emerging infectious disease vaccine candidates are built on the same rVSV vaccine platform. To address priority viruses that are likely to cause public health emergencies of international concern, our multidisciplinary expert teams and partners have developed and clinically advanced rVSV chimeric vaccine candidates: 1) VSVΔG-MARV-GP targeting Marburg virus (MARV) surface glycoprotein (GP), 2) VSVΔG-SUDV-GP targeting Sudan ebolavirus (SUDV) GP, and 3) VSVΔG-LASV-GPC targeting Lassa virus (LASV) surface glycoprotein complex (GPC). Single administrations of VSV∆G-LASV-GPC, VSV∆G-MARV-GP and VSV∆G-SUDV-GP vaccine candidates provided complete rapid and long-term protection against LASV, MARV and SUDV diseases respectively, when tested in NHP challenge studies. Importantly, a single intramuscular vaccination with our VSV∆G-LASV-GPC (doses from 2x10^4 to 2x10^7 PFU) or rVSVΔG-SUDV-GP (doses from 2x10^6 to 2x10^8 PFU) in recent Phase 1 trials (NCT04794218 and NCT05724472) induced robust long-lasting cellular and humoral (binding and neutralizing) responses. Ultimately, we comprehensively characterized the innate and adaptive immune responses and identified common immune signatures triggered by a diverse portfolio of our rVSV-vectored vaccines. This knowledge on VSV vaccine-induced immunity could collectively advance development of additional lifesaving rVSV platform-based vaccines against viruses with pandemic potential.

W173. Follow-up Investigation of Borderline Results in QuantiFERON-TB Gold-plus Assay

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Abstract Text: Background: The QuantiFERON-TB Gold Plus (QFT-Plus; Qiagen, Hilden, Germany) assay is commonly used to screen for latent tuberculosis infection (LTBI), but borderline results (0.2–0.7 IU/mL) frequently exhibit inconsistency upon follow-up. We investigated the follow-up results of borderline TB1 and TB2 values in the QFT-Plus assay. Methods: Over a six-year period, 865 individuals who initially presented with borderline results (0.2–0.7 IU/mL) in either TB1 or TB2 were retrospectively analyzed. We evaluated the agreement and correlation between TB1 and TB2 values, alongside trends in conversion and reversion. Additionally, follow-up results for a subset of borderline cases were compared between QFT-Plus and the STANDARD E TB-Feron ELISA (TBF; SD Biosensor, Suwon, Korea). Results: Initial TB1 and TB2 borderline results showed weak agreement (Cohen's κ = 0.575, 95% CI 0.520–0.629) and moderate correlation (Spearman's ρ = 0.646, 95% CI 0.606–0.684; $P < 0.001$). The highest rate of variability for TB2 was observed during the primary follow-up (34.9%; 52/149); however, this trend declined across consecutive evaluations. Individuals with borderline values in both TB1 and TB2 exhibited significantly higher rates of reversion and conversion. Conversely, the presence of a low-negative result (< 0.2 IU/mL) in either tube was associated with high stability and minimal variability. Both assays showed comparable results for borderline follow-up. Conclusion: Integrating TB1 and TB2 results from the QFT-Plus assay could refine the interpretation of borderline results. Follow-up testing is recommended when borderline results occur in both tubes. Future studies should focus on defining clinical thresholds and optimal intervals for follow-up testing.

W174. ChuS-dependent Carbon Monoxide–producing Probiotics Isolated from Human Volunteers Reduce the Severity of Inflammatory and Infectious Diseases

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Abstract Text: Inflammatory, infectious, and autoimmune diseases share a common characteristic: dysregulated immune responses that lead to tissue damage and worsen clinical outcomes across a wide range of pathological contexts and disease etiologies. Carbon monoxide (CO), traditionally considered toxic, is now recognized as an endogenous immunomodulatory molecule with potent anti-inflammatory and cytoprotective effects when administered at microdose levels. However, safe strategies for sustained CO delivery remain a significant obstacle to its therapeutic application and clinical translation. In this study, we investigated whether naturally CO-producing probiotic bacteria of Chilean origin, derived from the human gut microbiota, could serve as a biological platform for endogenous CO delivery through a host-compatible and microbiota-driven mechanism. Commensal strains of Escherichia coli isolated from healthy Chilean volunteers were selected based on the presence of the chuS gene, a bacterial heme oxygenase believed to be responsible for CO production and conserved among specific commensal lineages. The chosen strains (UCPROB) were fully sequenced to rule out transmissible antibiotic resistance and genotoxicity and showed probiotic properties *in vitro* and *in vivo* consistent with established safety and functionality criteria for probiotic candidates. Oral administration of UCPROB in C57BL/6 mice was safe and induced reproducible increases in carboxyhemoglobin, confirming CO production *in vivo* without adverse physiological effects or signs of systemic toxicity. Functionally, UCPROB treatment reduced disease severity, inflammatory markers, microbial dissemination, and tissue damage across multiple murine models, including viral, bacterial, intestinal inflammation, and autoimmunity, resulting in improved clinical and pathological outcomes. Notably, the use of chuS-deficient mutants demonstrated that the protective effects observed in selected models were dependent on bacterial CO production thereby establishing a direct link between probiotic-derived CO and disease attenuation. Taken together, these findings identify CO-producing probiotics of Chilean origin isolated from the human gut microbiota as a safe and effective strategy for reducing the severity of inflammatory, infectious, and autoimmune diseases with translational relevance. This work was supported by the Millennium Institute on Immunology and Immunotherapy, FONDEF IDeA grant ID20I10082, FONDECYT Regular grants 1231905, 1231851, and 1240971, and the ANID National PhD Fellowship No. 21251772.

W175. Machine Learning Analysis of Post-Acute COVID Symptoms Identifies Symptom Clusters, Severity Groups, and Trajectories

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Abstract Text: The wide heterogeneity of self-reported symptoms has complicated Long COVID research. Disease phenotyping efforts have relied on symptom groupings defined by clinical intuition, while computational approaches remain challenging due to the curse of dimensionality: treating each symptom as an independent feature can render patients effectively unique and hinder robust clustering. Here, we developed a machine learning (ML) framework that applies topic modeling (TM) to analyze de-identified symptom questionnaires from four post-acute COVID-19 cohorts: the University of California, San Francisco (UCSF; n = 669), Icahn School of Medicine at Mount Sinai (ISMMS; n = 615), Emory University (n = 60), and the University Hospital of Wales, Cardiff (n = 317). TM-based analysis identified distinct symptom clusters and latent symptom signatures within each cohort. Through meta-analysis, we harmonized cohort-specific clusters into ten global symptom meta-clusters and used cluster-level topic scores to construct a unified severity framework. Across all cohorts, three discrete severity levels: mild, moderate, and severe, emerged at the cluster level, with clear quantitative separation between groups. These ML-derived severity scores were significantly correlated with independent clinical and biological measures, including patient-reported health status (EQ-5D) and SARS-CoV-2–specific antibody responses measured in plasmablasts. Notably, a severe symptom cluster characterized by neurological and sex-hormonal manifestations, with a predominance of female patients, was reproducibly observed in both the UCSF and ISMMS cohorts. Several symptom clusters were also associated with relevant pre-existing conditions, including immunodeficiency and inflammatory bowel disease. Longitudinal analysis at the symptom level revealed three distinct trajectories: acute symptoms that resolve, symptoms that persist but attenuate, and progressively worsening symptoms. At the cluster level, two trajectories were identified during the acute phase (≤30 days post onset): recovery during the early post-acute phase (30–90 days post onset) or progression to Long COVID (>90 days post onset). Individuals exhibiting non-mild symptom clusters during the acute phase had a 2.6-fold increased risk of developing moderate or severe Long COVID compared with those presenting with mild acute symptoms. Together, these findings demonstrate that ML-based analysis of symptom questionnaires enables scalable, data-driven patient stratification and provides a foundation for precision management strategies in Long COVID.

W176. Thrombospondin-1 Mediates IL-10–mediated Myeloid Regulation to Protect the Lung During Pseudomonas Aeruginosa Infection

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Abstract Text: Pseudomonas aeruginosa is a major cause of hospital- and ventilator-associated pneumonia, in which bacterial virulence factors and dysregulated host inflammation contribute to extensive lung damage. Neutrophils and monocytes are essential for antimicrobial defense in the infected lung, yet excessive or poorly controlled activation of these myeloid cells can exacerbate tissue injury and compromise host survival. Thrombospondin-1 (TSP-1) is a host-derived glycoprotein previously shown to limit neutrophil-driven pathology during pulmonary P. aeruginosa infection. Here, we identify a broader immunoregulatory role for TSP-1 in shaping anti-inflammatory responses in the lung. Using a murine model of infection, we show that TSP-1 promotes the induction of interleukin-10 (IL-10) in lung tissue following P. aeruginosa challenge. Neutrophils and $Ly6C^+$ monocytes were the predominant sources of IL-10 during the first 48 hours post-infection, and this cytokine was essential for host survival, effective bacterial control, and attenuation of lung inflammation and injury. Mechanistically, *in vitro* studies revealed that TSP-1 drives IL-10 production in lipopolysaccharide-stimulated $CD11b^+Ly6G^+Ly6C^+$ cells differentiated from bone marrow precursors through a pathway dependent on the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ). Importantly, intratracheal adoptive transfer of IL-10–sufficient $CD11b^+Ly6G^+Ly6C^+$ cells into $Thbs1^{-/-}$ mice prior to infection restored pulmonary IL-10 production, enhanced host defense, and reduced lung inflammation and vascular permeability. Collectively, these findings demonstrate that TSP-1 coordinates a protective myeloid cell program by inducing IL-10 in neutrophils and monocytes, thereby balancing antimicrobial immunity with the prevention of excessive lung inflammation during P. aeruginosa pneumonia.

W177. Therapeutic Strategies Targeting Respiratory Syncytial Virus–Associated Disease: Monoclonal Antibody and Lipid Metabolic Approaches

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Abstract Text: Human respiratory syncytial virus (hRSV) is a major global health concern and a leading cause of severe lower respiratory tract infections, particularly in preterm infants. Beyond respiratory disease, hRSV can access the central nervous system (CNS), inducing acute and long-term neurological alterations. Despite its clinical burden, no approved vaccines exist for neonatal populations, highlighting the need for alternative therapeutic strategies. Currently licensed monoclonal antibodies (mAbs) against hRSV target the viral fusion protein (F-hRSV). Here, we evaluated the hRSV nucleoprotein (N-hRSV) as a therapeutic target. We generated a humanized anti-N-hRSV mAb, yielding four clones (P1-04H, P1-05D, P2-01A, and P2-01D), characterized through affinity, pharmacokinetic, and *in vivo* protection studies in BALB/c mice. All clones showed high affinity for N-hRSV protein, as determined by surface plasmon resonance, and two antibodies remained detectable *in vivo* for up to 30 days. Prophylactic administration of anti-N-hRSV mAbs significantly reduced weight loss, clinical scores, and viral load in lung and brain tissues. Behavioral test analyses 30 days post-infection revealed protection against long-term neurological alterations, including normalized anxiety-like and exploratory behaviors. *In vitro* studies demonstrated antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Additionally, to investigate mechanisms underlying hRSV-induced neurological pathology, we examined the effects of hRSV on CNS cells, focusing on neutral lipid metabolism. hRSV infection altered lipid composition and gene expression, while inhibition of lipid metabolism pathways reduced viral replication. Together, these findings identify anti-N-hRSV mAbs and host lipid metabolism as promising antiviral targets addressing respiratory and neurological manifestations of hRSV infection.

W178. B Cell Analysis with Multiplexed Antigen Panels Defines Determinants of Antibody Breadth in Human Vaccination and Infection

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Abstract Text: The cellular, immunogenetic and antigenic factors affecting the breadth of viral antigen variants recognized by human antibody responses are poorly defined. We developed highly multiplexed panels of DNA-tagged SARS-CoV-2 antigens from up to 20 viral variants to label and sort 6,262 antigen-binding circulating B cells from previously naïve mRNA vaccinees or infected patients, and from deceased organ donor lymphoid tissues, to enable antigen receptor and transcriptome sequencing. We applied quantitative modelling to evaluate phenotypic and BCR features associated with variant antigen binding breadth, and with infection versus vaccination responses. Blood from infected and vaccinated patients' showed the greatest enrichment of antigen-specific B cells in a class-switched memory population that expressed genes associated with the germinal centre, including somatic hypermutation enzyme AID and IL-21 receptor, suggesting recent emigration. Antigen specificity was also enriched in atypical B cells that expressed CD11c and T-bet that have been previously reported to transiently expand during CoV-2 infection and are proposed to be B cells stimulated at extrafollicular sites outside GCs. In contrast to atypical B cells, post-germinal centre B cells showed progressively increasing variant binding breadth and somatic hypermutation over time and enriched usage of V-gene IGHV3-53 and isotype IgG1. Vaccination compared to

infection possessed greater variant binding breadth, with infection preferentially stimulating atypical B cells. This large-scale analysis reveals key determinants of antigen-binding breadth, critical for understanding responses to viral infection and guiding vaccine development against rapidly mutating viruses.

Mucosal Immunology

Th180. Antigenic Cross-Roads: Viral–Self Mimicry Drives CD8$^+$ T Cell–Mediated Epithelial Injury in Ulcerative Colitis

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Abstract Text: Aberrant CD8$^+$ T cell responses to self, commensal, or environmental antigens are thought to drive ulcerative colitis (UC), yet no antigenic target has been identified. Here, we identify viral–self cross-reactivity as a mechanistic axis linking infection, immune dysregulation, and tissue-specific autoimmunity in UC. We show that virus-specific CD8$^+$ T cells—particularly EBV-reactive clonotypes—selectively expand in UC and cross-recognize the epithelial tight-junction protein Claudin-2, providing the first direct evidence that antiviral immunity can be redirected toward epithelial self-antigens in UC. Using an integrated single-cell TCR/transcriptomic, antigen-mapping, spatial profiling, and organoid-based functional pipeline spanning the appendix, colon, and draining lymph nodes, we show that these antigen-specific CD8$^+$ T cells acquire a pathogenic GZMK$^+$ effector program capable of compromising barrier integrity in patient-derived colonic organoids. We further demonstrate that their colonic accumulation is appendix-dependent: UC patients with prior appendectomy lack this GZMK$^+$ effector population, and its loss cannot be compensated by other lymphoid tissues. Moreover, the loss of this effector axis is associated with epithelial restitution, providing a mechanistic explanation for why appendectomy reduces UC risk and promotes endoscopic remission even in therapy-refractory patients. Collectively, our results transform molecular mimicry from a theoretical model into a validated mechanism of human UC, integrating long-standing epidemiological observations—including viral associations, the protective effect of appendectomy, and epithelial barrier defects—into a unified mechanistic framework. They also reveal antigen-specific CD8$^+$ T cell pathways as tractable targets for precision therapies beyond broad immunosuppression.

Th181. Skeletal Muscle DUSP29/DUPD1 Links Colitis with Obesity-associated Metabolic Diseases

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Abstract Text: Objective of the study: Inflammatory bowel disease (IBD), comprising of Crohn's disease and Ulcerative Colitis (UC), is a debilitating inflammatory disease of the gastrointestinal tract with no known cause. Although extensive work has linked numerous genetic loci to IBD, most of these associations remain poorly understood. In addition, some studies have associated IBD to metabolic diseases such as diabetes and non-alcoholic fatty liver disease (NAFLD), although the precise molecular and pathophysiological mechanisms underlying these associations are unknown. Thus, the central objective of this study was to understand the molecular mechanisms underlying the associations between IBD and obesity related metabolic diseases. Methods and Results: Employing multiple murine models of colitis, colitis-associated colon cancer (CAC) and obesity-associated metabolic diseases, we have identified DUSP29/ DUPD1, a phosphatase of unknown function, that links IBD with metabolic diseases. Specifically, Dupd1$^{-/-}$ mice are protected from dextran sodium sulfate (DSS) and Helicobacter hepaticus induced colitis, and DSS/azoxymethane (AOM) induced CAC. Dupd1$^{-/-}$ mice also exhibited protection against high fat diet (HFD) induced obesity and non-alcoholic fatty liver disease (in female mice) and glucose

intolerance in both male and female mice. DUPD1 is exclusively expressed in the skeletal muscle. Indeed, using skeletal muscle specific conditional DUPD1 knockout mice (Dupd1fl/fMyf6Cre), we show that DUPD1 exerts its colitogenic and obesogenic effects from the skeletal muscle through modulation of autophagy, mitochondrial function and mitophagy implicating all these processes in DUPD1 mediated IBD and metabolic diseases. These data also demonstrate a novel role of skeletal muscle in promoting inflammation in distant organs such as the colon, a finding that suggests a paradigm shift in IBD research. Importantly, a dual specificity phosphatase inhibitor (NSC-663284) inhibited DUPD1 enzyme activity, modulated autophagy flux and reduced DSS-induced colitis in wildtype mice but not in Dupd1$^{-/-}$ mice. Conclusions: Our study identifies Dusp29/Dupd1 as a central gene that links IBD and obesity-associated metabolic diseases and suggests that it is a new therapeutic target in IBD, and obesity-associated metabolic diseases.

Th182. Harnessing Next-generation Phage Therapy Against Graft-versus-host Disease

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Abstract Text: Acute graft-versus-host disease (aGVHD) is a severe, potentially fatal complication following allogenic haematopoietic cell transplantation (allo-HCT) for haematological disorders. Patients undergoing allo-HCT face numerous clinical interventions—including chemotherapy, radiation, and antibiotic treatments—that influence the development of aGVHD. Growing evidence highlights the crucial role of the intestinal microbiome in aGVHD pathogenesis. Early studies in the 1970s demonstrated that mice subjected to allo-HCT under germ-free conditions exhibited a reduced incidence of GVHD. With advancements in microbial analysis techniques, clinical studies have since revealed that disruptions to the intestinal environment, such as dysbiosis, are closely linked to the manifestation and severity of aGVHD symptoms. Despite this knowledge, effective and safe strategies to restore gut microbial balance remain undeveloped. Recent research has identified the expansion of intestinal Enterococcus faecalis as a key dysbiosis-related risk factor for aGVHD. Our analysis of the intestinal microbiome in allo-HCT patients revealed that E. faecalis evades elimination and proliferates by forming biofilms, rather than through acquiring antibiotic resistance genes. From patient fecal samples, we isolated highly pathogenic, cytolysin-positive E. faecalis strains. Whole genome sequencing of these bacteria enabled the identification of an anti-E. faecalis enzyme derived from bacteriophages specific to E. faecalis. This phage-derived antibacterial enzyme demonstrated potent lytic activity against E. faecalis biofilms both *in vitro* and *in vivo*. Further evaluation using aGVHD-induced gnotobiotic mice colonized either with E. faecalis or with patient fecal samples dominated by Enterococcus showed that treatment with the E. faecalis-specific enzyme significantly reduced intestinal Enterococcus populations and improved survival rates compared to untreated controls. These findings suggest that targeting biofilm-forming, pathogenic E. faecalis with phage-derived antibacterial enzymes offers a promising therapeutic approach for preventing or mitigating aGVHD, especially since these bacteria are difficult to eradicate using conventional antibiotics. In summary, the discovery and application of a bacteriophage-derived enzyme targeting E. faecalis biofilms represent a novel strategy to address gut dysbiosis-associated risks in allo-HCT patients. This approach could enhance protection against aGVHD by specifically eliminating a critical pathogenic component of the intestinal microbiome that conventional treatments fail to control.

Th183. Dietary Antigens Gradually Inactivate CD4⁺ T Cells to Produce Oral Tolerance

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Abstract Text: CD4 T cells specific to dietary antigens are inactivated through mechanisms that include deletion, anergy, and acquisition of regulatory capacities. The factors that guide the fate of these cells are unknown. Here, we analyzed the role of antigen-associated factors (e.g., concentration, frequency, affinity) in determining CD4 T cell fate during oral tolerance. We adoptively transferred naïve OTII CD4 T cells and fed mice with different regimes of the model antigen ovalbumin (OVA). A single dose of OVA induced an early but short-lived induction of FoxP3. Gradually, FoxP3⁺ cells decreased and anergic cells became the dominant population. Fate mapping analyses revealed that regulatory and anergic programs are mostly exclusive. Ultimately, the majority of OTII cells became apoptotic. Repetitive administration of OVA was associated with a higher expression of anergy-associated molecules (e.g., Ctla-4, Nrp-1) and transcription factors (e.g., TOX, Helios) in a dose-dependent manner, suggesting that repetitive exposure to cognate antigen gradually induces a more profound CD4 T cell inactivation. Additionally, repetitive exposure to OVA promoted homing of anergic CD4 T cells into the lamina propria. However, tolerance was a short-lived process, as its effect was not detected after OVA was discontinued. Using OVA-specific tetramers, we identified high and low affinity CD4 T cells and explored the transcriptomic changes induced by oral antigen (scRNA-seq). *In vitro* examination of tolerogenic fates using ligands of different affinities showed that weak TCR activation was associated with the development of anergy. Collectively, our results indicate that tolerance to oral antigens represents a gradual and dynamic process, controlled by the intensity of antigenic stimulation, where expression of FoxP3 and anergy-associated markers represent a transient phase that leads to clonal deletion.

Th184. Breast Milk-derived Signals Regulate Epithelial Cells Maturation and Gamma Delta-IEL Colonization to Shape Early Life Intestinal Homeostasis

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Abstract Text: The intestinal epithelium is a specialized barrier that balances tolerance and defense against luminal antigens and microbes. Its maturation during early life is shaped by intraepithelial lymphocytes (IELs), the microbiota, and maternal factors. Natural CD8αα⁺ αβ and Vγ7⁺ γδ IELs are critical for epithelial homeostasis, repair, and microbial regulation, yet the mechanisms controlling their postnatal maintenance remain poorly understood. We combined flow cytometry, cross-fostering and co-housing experiments, adoptive IEL transfer into RAG⁻/⁻ hosts, intestinal epithelial cell RNA sequencing, and unbiased proteomic analysis of milk from Taconic (Tac) and Jackson (Jax) dams to dissect microbiota-dependent and -independent mechanisms controlling IEL development and epithelial maturation during early life. Taconic mice showed markedly fewer natural CD8αα⁺ and Vγ7⁺ γδ IELs throughout the small intestine (SI), while colonic frequencies remained comparable to Jackson mice, indicating a compartmentalized defect. CD8αα⁺ IEL loss was microbiota-dependent, as co-housing with Jax mice restored their abundance, whereas Vγ7⁺ IEL loss was microbiota-independent and emerged at weaning (day 28) despite normal thymic output. Cross-fostering Tac pups onto Jax dams fully rescued Vγ7⁺ IELs, implicating milk-derived factors. Proteomic profiling of Tac versus Jax milk revealed broad protein reductions, notably in IGF transport and uptake pathways, with decreased IGF-1 and osteopontin. Adoptive transfers showed a tissue-intrinsic defect, as Tac IELs engrafted in Jax RAG⁻/⁻ hosts but not vice versa. SI epithelial transcriptomics revealed downregulation of c-Myc targets after day 28, coinciding with Vγ7⁺ IEL loss and defective epithelial maturation. Together, these findings reveal that postnatal maintenance of natural IELs depends on distinct environmental cues: while CD8αα⁺ IELs are shaped by the microbiota, Vγ7⁺ γδ IELs require maternal milk–derived factors that support epithelial maturation and

c-Myc–dependent pathways. This identifies a critical early-life window in which deficient milk signals can impair intestinal immune–epithelial development and long-term mucosal homeostasis.

Tu179. Limosilactobacillus Fermentum SRK414 Drives Epithelial–macrophage Immunometabolic Crosstalk in Intestinal Inflammation

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Abstract Text: Intestinal inflammation is sustained by epithelial barrier dysfunction and pro-inflammatory macrophage activation. Effective resolution likely requires coordinated immunometabolic adaptation across epithelial and immune compartments. Limosilactobacillus fermentum SRK414, isolated from a healthy infant, was evaluated for its capacity to modulate this axis in intestinal inflammation. Acute colitis was induced using 2% dextran sulfate sodium (DSS), followed by therapeutic oral administration of SRK414 (1×10^9 CFU/mL). Immune populations were analyzed by flow cytometry. Mechanistic studies were performed in Caco-2 and HT-29 epithelial cells and in phorbol 12-myristate 13-acetate (PMA)–differentiated THP-1 macrophages. Untargeted metabolomics and whole-genome sequencing were used to identify metabolite-associated pathways. In DSS-induced colitis, SRK414 significantly reduced disease activity and histopathologic injury. In peritoneal cavity cells, $CD11b^+F4/80^+$ macrophages showed reduced $Trem1^+$ M1 macrophages and increased $CD206^+$ M2 macrophages populations. In splenocytes, $Foxp3^+$IL-10^+ regulatory T cells expanded. Colonic tissue exhibited decreased expression of inflammatory mediators (Tnf, Il1b) and increased Il10 and junction–associated genes (Cdh1, Tjp1), indicating recovery of mucosal barrier–immune balance. In epithelial cells, SRK414 supernatant enhanced junction–related gene expression and induced metabolic alterations characterized by enrichment of itaconic acid, a regulator of macrophage immunometabolism, and 3-(imidazol-5-yl)lactate, an aryl hydrocarbon receptor (AhR)-associated metabolite, in conditioned media. In PMA-differentiated THP-1 macrophages, SRK414 promoted M2 polarization and suppressed lipopolysaccharide-induced TNFα and IL-6 while enhancing IL-10 production. AhR activation was observed in epithelial reporter assays, consistent with partial involvement of metabolite-sensing pathways in barrier restoration. Together, these findings support a microbe-driven immunometabolic mechanism linking epithelial metabolic remodeling to macrophage plasticity and regulatory T cell expansion in intestinal inflammation, highlighting a role for microbial metabolites in coordinating epithelial–immune crosstalk during mucosal recovery.

Tu180. Secretoglobin (SCGB) - An Under-appreciated Mucosal Defense Protein Family

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Abstract Text: Secretoglobins (SCGB) are mammalian, secreted small alpha helical proteins. There are 11 known human SCGB proteins which are found in a variety of different secretion types and locations across the body. These secreted proteins are often found in the mucosa at barrier defense interfaces such as the lungs, mouth, skin, reproductive organs, among others. By reviewing what's currently known about the 11 known human SCGBs, we see that different SCGBs' expressions have implications in a variety of different immunological roles including bacterial defense, cancers, and inflammation, to name a few. Through GWAS, PheWAS, *in vitro* bacterial growth assay, and *in vivo* murine data, we show that SCGB1D2 inhibits growth of the bacteria that causes Lyme disease and affects susceptibility to Lyme disease. Several other SCGBs have been found to have microbial defense properties, including findings in LPS neutralization as well as development and function of alveolar macrophages, which are crucial in respiratory pathogen defense. These findings suggest that the SCGB protein family plays a larger role in host mucosal barrier defense against pathogens, is likely under-appreciated in immunology, and has therapeutic potential as a novel drug class.

W179. A JAK-STAT-activated Myeloid Hub at Ulceration Sites Underlies Resistance to Anti-TNF Therapy in Crohn's Disease

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Abstract Text: Understanding the molecular basis of immunotherapy resistance in immune-mediated inflammatory diseases including inflammatory bowel disease (IBD) is key to preventing irreversible tissue damage. Because myeloid cells drive Crohn's disease (CD) pathogenesis, we hypothesized that distinct myeloid states shape disease progression and treatment response. Using integrative single-cell and molecular analyses, we uncovered previously unrecognized axes of monocyte–macrophage (mo-mac) heterogeneity in the inflamed ileum defined by combinations of transcriptional programs in refractory ileal CD. These programs and mo-mac states were validated in several external cohorts. A pathogenic mo-mac subset, interferon-associated inflammatory monocytes (IFIM), defined by convergent NF-κB and IFNγ signaling, dominated late-stage disease. Baseline IFIM enrichment predicted anti-TNF resistance and severe postoperative recurrence, with IFIM-derived ligands sustaining epithelial destruction. IFIM showed JAK–STAT activation, indicating susceptibility to JAK inhibition (e.g., upadacitinib). Thus, IFIM-associated inflammation underlies anti-TNF-resistant CD and provides a framework for molecular patient stratification and mechanism-guided therapy.

W180. Intestinal Influence on Exocrine Pancreatic Immunity and Cancer Development via IL-22

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Abstract Text: IL-22 is a pleiotropic cytokine that is known to induce the expression of anti-microbial genes, including the Reg gene family, in the intestine as well as direct a tissue repair program. The pancreas is functionally and anatomically related to the intestine and has been shown to be highly sensitive to IL-22. While elevated IL-22 has been associated with several pancreatic diseases including pancreatic ductal adenocarcinoma (PDAC) and diabetes, the precise transcriptional targets of IL-22 signaling in this organ have not yet been determined. Here, we set out to comprehensively characterize the transcriptional program induced by IL-22 in the pancreas and gut of both mice and human using bulk RNA sequencing. To do this in mice, we employed a transgenic mouse model in which Il22 is under the control of the albumin promoter in the liver, resulting in systemically high concentrations of IL-22 in serum. For human, we developed a pancreatic explant system for *ex vivo* culture of human pancreas and used intestinal organoids to determine IL-22 targets in the gut. This analysis revealed that Reg genes were the most upregulated genes in the murine exocrine pancreas in response to IL-22. Interestingly, IL-22 decreased the expression of many metabolic genes as well as genes encoding for digestive enzymes, suggesting that IL-22 can modulate the function of the exocrine pancreas. We also investigated whether gut microbes could modulate pancreatic gene expression via IL-22 in mice. We found that colonization with both commensal and pathogenic IL-22 inducing gut microbes resulted in significant changes in IL-22 regulated gene expression in the exocrine pancreas. Finally, we determined that elevated IL-22 accelerates PDAC progression in a murine model of the disease. Overall, we have defined the transcriptional program induced by IL-22 in the exocrine pancreas and intestine of mice and human and identified gut microbes as key regulators of pancreatic gene expression via IL-22 induction. Thus, this

work may provide mechanistic insight into both the homeostatic and disease promoting functions of IL-22 in the pancreas.

W181. Oral Mucosal Neutrophils in SARS-CoV-2 Infection Are Defined by an Interferon Signature and Restrained Heterogeneity

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Abstract Text: Cell response states to viruses in the oropharyngeal cavity is an area of emerging investigation. This is especially true for the antiviral response of neutrophils, which are key players in mucosal immunity. Whether neutrophils are beneficial in the antiviral response may be context dependent (e.g., mucosal site, virus). Here, we sought to characterize the cellular state of neutrophils in response to infection with SARS-CoV-2. To accomplish this, single-cell RNA sequencing was performed (Honeycomb HIVE CLX) on cells captured from gingival mucosal swabs of patients infected with SARS-CoV-2 or uninfected controls. Analysis was performed using a standard Seurat workflow (Version 5). Unsupervised clustering revealed six distinct clusters of neutrophils across groups, functionally characterized by the relative expression levels of cytokines (IL1B, IL8) and calprotectin (S100A8/A9). Individuals infected with SARS-CoV-2 were found to have a predominance of neutrophils expressing high amounts of IL8 (CXCL8) and relatively lower levels of calprotectin and IL1B. This contrasts with the uninfected controls, where the neutrophil population was more evenly represented by a clusters defined by either relatively high expression of IL1B and IL8 or relatively high expression of calprotectin. This clustering also revealed a profound interferon signature that dominates nearly all neutrophils of the SARS-CoV-2 infected individuals. Gene set enrichment analysis at the pseudobulk level further revealed a positive enrichment in pathways involved in the antiviral and interferon response. Taken together, these data implicate oral mucosal neutrophils in the immune response to SARS-CoV-2 and form initial steps towards further understanding the role of the oral cavity in viral clearance vs. systemic dissemination.

W182. Distinct Mast Cell States Across Human Mucosal Niches Identified by Single-Cell RNA Sequencing

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Abstract Text: Distinct Mast Cell States Across Human Mucosal Niches Identified by Single-Cell RNA Sequencing Authors: Katelyn White1, Michelle Galeas-Pena1, Brandy Sullivan1, Daniel W. Irelan1, Fathima N. Ismail1, and Sarah C. Glover1 Section of Gastroenterology. School of Medicine, Tulane University , New Orleans, LA, United States Mast cells (MCs) are bone marrow-derived granulocytes that play key roles mediating allergic responses and in regulation of innate and adaptive immunity. Although MCs are broadly distributed across mucosal surfaces, whether their cellular states differ according to tissue context remains unclear. To address this question, we analyzed publicly available single-cell RNA sequencing datasets from healthy adult subjects (ages 18-60) encompassing multiple mucosal tissues, including the oral mucosa, gingiva, nasal mucosa and duodenum. Using an integrated single-cell analysis framework, we identified mast cell populations and compared their transcriptional profiles across tissues. Our analysis revealed distinct, tissue-specific mast cell states characterized by differential expression of genes involved in immune activation, signaling, and tissue interaction. These findings demonstrate that mast cells exhibit marked transcriptional heterogeneity across mucosal niches, consistent with functional specialization driven by local environmental cues. Together, this work highlights the importance of tissue context in shaping mast cell identity and provides a framework for understanding how mast cell heterogeneity may contribute to site-specific immune responses at mucosal barriers.

W183. Regulatory T cells Augment Human Intestinal Epithelial Stem Cell Function Through a PDGF-AA–dependent Pathway

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Abstract Text: Regulatory T cells (Tregs) maintain immune tolerance via their TCR-dependent functions; however, their direct contribution to tissue repair remains sparse, with most evidence being limited to murine models. To define human Treg intestinal tissue-specific mechanisms, we established epithelial stem cells-only patient–derived human intestinal organoids (enteroids) from Crohn's disease and co-cultures them with primary thymic Tregs and lentiviral-mediated FOXP3–engineered Treg cells (CD4LVFOXP3). Conventional activated $CD4^+$ T cells disrupted barrier integrity, increased apoptosis, and depleted $LGR5^+$ stem cells. In contrast, CD4LVFOXP3 and thymic Tregs preserved stemness, enhanced enteroid size, and improved barrier function. Multiplex secretome profiling of coculture supernatants demonstrated a FOXP3-dependent reduction in pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1β) alongside enrichment of growth- and tissue-repair–associated factors (VEGF, PDGF-AA, IL-22, amphiregulin). Neutralizing antibody–mediated and pharmacological inhibition of PDGF-AA–PDGFRα signaling impaired $LGR5^+$ stem cells' growth and barrier function, identifying this pathway as a key regenerative axis mediated by $FOXP3^+$Tregs. These findings delineate a direct, human tissue-intrinsic role for $FOXP3^+$ Treg programs in epithelial regeneration and demonstrate that both naturally occurring and engineered Tregs support barrier integrity in addition to their T Cell specific immunosuppression. By separating epithelial effects from stromal and myeloid influences, this experimental framework deconvolutes Treg–stem cells' crosstalk and reveals novel therapeutic targets. Collectively, the data provide a rationale for using CD4LVFOXP3 to restore epithelial integrity in Crohn's disease and highlight PDGF-AA–PDGFRα signaling as a tractable pathway to sustain human intestinal stem cells.

Neuroimmunology

Th187. The Role of Mast Cells and MrgprB2/MrgprX2 in Pain Modulation

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Abstract Text: Mast cells play a crucial role in pain modulation, particularly through the Mas-related G-protein coupled receptor B2 (MrgprB2) in mice, the equivalent of the human MrgprX2 gene. These receptors, predominantly expressed on mast cells, are involved in responding to various stimuli, enhancing pain perception in conditions like osteoarthritis (OA). Increased mast cell populations in OA-affected joints contribute to inflammation and pain. In our *in vivo* studies, MrgprB2 knockout (KO) mice were subjected to destabilization of the medial meniscus (DMM), resulting in an increased OA phenotype and mast cell population compared to control-DMM mice. Notably, MrgprB2 KO-DMM mice exhibited reduced pain sensitivity, suggesting a complex role for MrgprB2 in pain perception. Further analysis revealed that in the brain, MrgprB2 KO-DMM mice showed decreased expression of pain-related proteins TRPV1 and GFAP, along with changes in neuron-specific beta III tubulin morphology. These results underline the importance of MrgprB2/MrgprX2 in modulating pain and point to these receptors as potential therapeutic targets for pain relief in chronic inflammatory diseases. Future research should aim to develop specific inhibitors for MrgprB2/MrgprX2, exploring their potential to reduce pain while preserving essential mast cell functions.

Th188. Large-scale Proteomic Profiling of Human Multiple Sclerosis Cortices Uncovers Vascular and Blood–brain Barrier–related Protein Changes

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Abstract Text: Disruption of the blood–brain barrier (BBB), allowing infiltration of blood-borne proteins such as the coagulation factor fibrin, is a key feature of multiple sclerosis (MS) pathogenesis. Fibrin promotes innate immune activation, including pathogenic microglial activation leading to inflammation and neurodegeneration. Evidence for BBB disruption in normal-appearing grey matter (NAGM) is controversial, with some studies reporting no plasma protein leakage, while others demonstrate cortical fibrin deposition in progressive MS associated with neuronal loss. To systemically assess protein changes beyond focal lesions in MS, we performed Tandem Mass Tag (TMT) proteomics on post-mortem frozen normal-appearing dorsolateral prefrontal cortices (DLPFC) of 261 patients with MS (pwMS) and 353 non-neurological control patients from five different brain banks. Differential abundance analysis of the pre-processed protein abundance matrix between pwMS and control patients was performed by fitting a linear model adjusting for age, sex and white matter fractions and revealed a total of 540 upregulated and 594 downregulated proteins in MS. Among the proteins showing the largest effect sizes were multiple proteins consistent with increased vessel permeability, including fibrinogen peptides, defensins, CRP as well as several immunoglobulin chains. Moreover, elevated levels of MMP9 and ITGA7 as potential markers of basement membrane and perivascular remodeling were observed in pwMS. Future work will include validation by histological assessment of vascular integrity in the same tissue samples. Such validation will be important for informing future therapeutic strategies aimed at modulating fibrin-associated innate immune activation and its potential neurotoxic effects in pwMS.

Tu181. CNS-derived Antigens Promote Tregs That Redefine Immune Privilege and Regulate Peripheral Immune Responses

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Abstract Text: The central nervous system (CNS) has long been regarded as “immune privileged,” with immune responses within the parenchyma presumed to be limited. The rediscovery of meningeal lymphatics and specialized immune niches at CNS borders has challenged this view, raising the question of whether brain-derived antigens actively shape systemic immunity. Revisiting classical transplantation paradigms, we demonstrate that CNS immune privilege reflects active immune education rather than immune ignorance. Peripheral priming with a skin allograft accelerates rejection of a subsequent intracerebral graft, indicating that brain-implanted tissues are immunogenically recognized. In contrast, in unprimed hosts, intracerebral skin grafts initially survive and vascularize but are ultimately rejected. Furthermore, prior intracerebral priming delays rejection of a later peripheral skin graft, revealing a dominant tolerogenic program initiated by CNS antigen exposure with systemic consequences. Mechanistically, we identify regulatory T cells enriched at dural sinuses—termed CNS border Tregs (bTregs)—that persist independently of microbiota or dietary antigens. Delivery of CNS-derived peptides via cerebrospinal fluid converts antigen-specific naive T cells into bTregs through a TGF-β– and CNS1-dependent pathway. Genetic disruption of CNS1 selectively reduces dural Tregs, enhances cytotoxic immune infiltration, and accelerates intracerebral allograft rejection. Functionally, intracisternal magna delivery of MHC class II–restricted neoantigens expands antigen-specific bTregs and abolishes the efficacy of peripheral neoantigen vaccination against tumors expressing the same epitopes. This systemic immunosuppression is reversed when bTreg generation is impaired. Together, these findings redefine CNS immune privilege as an active, border-centric tolerogenic program that shapes peripheral immunity, with implications for transplantation, neuroimmunology, and cancer immunotherapy.

Tu182. CD8+ T cell Antigen Recognition Reveals STAT5B Trends in Narcolepsy Type 1

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Abstract Text: Narcolepsy type 1 (NT1) is caused by the loss of hypocretin (HCRT) producing neurons in the lateral hypothalamus. It is associated with HLA DQ06:02, present in 98% of cases, TCR genes TRAJ24, TRAJ28, and TRBV4-2, and to a lesser extent, HLA A11:01. Environmental events, such as influenza/A H1N1 (2009 pandemic H1N1) infection and vaccination, have been linked to NT1 onset. The genetic and environmental findings support a model in which NT1 arises through T cell-mediated autoimmunity triggered by prior influenza exposure. Antigen-specific CD8+ T cells have been identified, notably targeting intracellular RFX4 and LHX9 epitopes presented by various HLA class I alleles; however, their recognition patterns and role in disease are unclear. A panel of 1057 peptides derived from influenza and HCRT-specific proteins restricted to HLA A02:01 and A11:01 were predicted with NETMHCpan4.1. DNA barcoded pMHC class I multimers were built with the peptide panel and used to stain PBMC samples from 24 HLA A02:01+/A11:01+ NT1 patients and 23 matched controls. A phenotypic antibody panel was also used to stain the PBMC samples. Multimer-bound CD8+ T cells were FACS-sorted and DNA barcodes sequenced. Barcode sequence information was analyzed with Barracoda 1.8. NT1 patients and controls recognize similar numbers of viral peptides and at similar frequencies. Although both groups recognize similar numbers of self-peptides, the frequency of individual CD8+ T cell populations recognizing autoantigens is increased in NT1 patients. Controls recognize a greater number of peptides derived from the autoantigen STAT5B than NT1 patients. Despite this, the frequency of the CD8+ T cell populations recognizing STAT5B in NT1 patients is greater than controls. No differences in populations identified with the phenotypic antibody panel were observed between NT1 patient and control samples. NT1 patients exhibit fewer but higher-frequency STAT5B-specific CD8+ T cell populations, suggesting potential clonal expansion of self-reactive T cells. Like RFX4 and LHX9, STAT5B is an intracellular transcription factor enriched in HCRT neurons. Intracellular antigens may be common targets of CD8+ T cell responses in NT1. Functional studies are needed to characterize these CD8+ T cell populations and their relevance to disease.

Tu183. Cold-induced Immunomodulation Requires Proper Sympathetic Architecture Maintained by Endothelial Semaphorin 6D

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Abstract Text: Environmental temperature critically shapes host immunity, with cold conditions weakening infection defenses while aggravating autoimmune and allergic disorders. Cold exposure activates the sympathetic nervous system. Proper sympathetic nerve distribution in lymphoid organs has been shown to be important for optimal immune responses. However, the molecular mechanisms regulating sympathetic architecture and its impact on temperature-dependent immunity remain poorly understood. We recently found that semaphorin 6D (Sema6D), an axon guidance molecule, regulates sympathetic nerve architecture, as Sema6d-/- mice exhibit increased sympathetic nerve density in lymph nodes. We therefore examined whether Sema6D mediates cold-induced immune alterations through regulation of sympathetic nervous system architecture. Experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis, was induced in wild-type and Sema6d-/- mice, including global and cell type-specific knockout models, housed at room temperature (22°C) or cold (10°C). Disease manifestations, central nervous system pathology, and peripheral lymphoid responses were evaluated through immunophenotyping and histological analysis. At room temperature, wild-type and Sema6d-/- mice developed equivalent EAE pathology. However, cold-challenged Sema6d-/- mice demonstrated markedly diminished disease severity, reduced central nervous system inflammation, and decreased demyelination compared to controls. Endothelial-specific Sema6d deletion

(Sema6dΔVEcad) replicated this cold-specific protection, whereas neuronal or microglial-targeted deletions showed no effect. Adoptive transfer experiments confirmed that endothelial Sema6D governs antigen-specific $CD4^+$ T cell priming within lymph nodes during cold challenge. Mechanistically, Sema6dΔVEcad mice exhibited aberrantly increased perivascular sympathetic innervation density in the lymph node, despite normal vascular patterning. Under cold exposure, these mice showed elevated blood pressure and tissue norepinephrine levels. This was accompanied by intensified lymph node hypoxia and elevated cellular stress responses, including upregulated endoplasmic reticulum stress markers and oxidative stress factors in $CD4^+$ T cells. These microenvironmental changes resulted in reduced lymph node cellularity and impaired T cell responses. Notably, pharmacological sympathetic denervation using 6-hydroxydopamine reversed the cold-mediated disease attenuation in Sema6dΔVEcad mice, restoring EAE susceptibility to control levels, establishing causality. Our findings demonstrate that endothelial Sema6D regulates immune homeostasis under cold exposure through control of peripheral sympathetic nerve architecture. This work reveals how sympathetic innervation patterns influence the lymphoid microenvironment to modulate immune responses, suggesting potential therapeutic strategies for environmentally-influenced inflammatory conditions.

W184. Impaired Adaptive Astrocyte Senescence Disrupts Cell-cell Communication and Drives Stress Susceptibility in Major Depressive Disorder

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Abstract Text: Chronic stress is a risk factor for Major Depressive Disorder (MDD); however, significant individual variability exists in stress resilience. Elucidating the molecular basis of this resilience is crucial for understanding MDD pathology. While cellular senescence is widely recognized as a pathogenic driver of aging and inflammatory diseases, the Senescence-Associated Secretory Phenotype (SASP) can also mediate beneficial cell-cell communication essential for maintaining tissue homeostasis and plasticity. We hypothesized that physiological senescence plays a pivotal role in maintaining brain homeostasis under psychosocial stress, serving as a fundamental stress resilience capacity, and that the failure of this physiological senescence contributes to MDD susceptibility. Gene Set Enrichment Analysis of bulk RNA-seq datasets from postmortem brains of MDD patients revealed significant downregulation of senescence-associated gene signatures in the prefrontal cortex (PFC) compared to controls. To elucidate the causal link, we utilized the "Social Status Loss (SSL)" mouse model. In this model, dominant mice subjected to "Forced Loss (FL)" exhibiting depression-like behaviors displayed significant suppression of senescence-associated genes. Notably, a negative correlation was observed between the severity of depressive symptoms and senescence gene expression. Weighted Gene Co-expression Network Analysis (WGCNA) revealed that in mice exhibiting impaired induction of this physiological senescence, pathways related to cytokine-mediated signaling and leukocyte activation were enriched. Conversely, resilient mice that maintained physiological senescence showed enrichment in neurogenesis and glial cell differentiation pathways. Validation using the Chronic Social Defeat Stress (CSDS) model confirmed that resilient mice exhibited higher senescence enrichment scores, whereas susceptible mice showed lower scores. Interestingly, non-responders to selective serotonin reuptake inhibitors (SSRIs) also exhibited lower senescence scores compared to responders. Next, single-nucleus RNA-seq analysis of MDD postmortem brains identified significant downregulation of senescence-associated genes specifically in PFC astrocytes. Furthermore, astrocyte clusters characterized by a failure of physiological senescence exhibited diminished cell-cell communication and a marked collapse of intracellular transcription factor networks centered on specific nuclear receptors. Our findings challenge the conventional view of senescence. We propose that astrocyte senescence functions as an adaptive stress response, or beneficial senescence, necessary for stress resilience. Consequently, senescence insufficiency in astrocytes may represent a novel maladaptive mechanism underlying the pathogenesis of MDD.

W185. Skull Bone Marrow Niche Activation Mediates Peripheral-CNS Immune Crosstalk in Alzheimer's Disease

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Abstract Text: Background: Amyloid β (Aβ) accumulation is a hallmark of Alzheimer's disease (AD), yet Aβ-targeted therapies show limited efficacy, underscoring the need for alternative targets. Neuroinflammation is a third core AD pathology, involving both resident microglia and infiltrating peripheral immune cells. However, the mechanisms behind peripheral immune cell recruitment remain poorly defined. Recent work identifies the skull bone marrow (SBM) as a unique immune niche with direct channels to the central nervous system (CNS), suggesting a potential route for neuroimmune communication. Its role in AD has not been established. Our preliminary findings indicated that Reelin, a pro inflammatory endothelial factor, is elevated in AD and may reflect SBM activation. Here, we investigated whether SBM activation represents a novel axis linking CNS pathology to peripheral immune responses in AD. Methods: Plasma Reelin levels were quantified in AD patients and mouse models. To assess SBM activation, WT mice received PBS or $A\beta_{1-42}$ fibrils, followed by Reelin mRNA quantification via PCR, and immune composition of SBM niche profiling by flow cytometry. To test whether niche activation is modifiable, Reelin inhibition using the CR 50 antibody was evaluated in 5xFAD and APPNL-F mice, and SBM niche size and density quantified. Longitudinal 9.4T T_2 weighted MRI validated *in vivo* SBM niche changes following CR-50 treatment. Results: Plasma Reelin increased with disease severity in AD patients and mouse models. Reelin mRNA was selectively upregulated 2-fold in SBM, decreased in femur, and unchanged in liver, mirroring plasma levels. $A\beta_{1-42}$ fibril injection elevated plasma Reelin and induced SBM-specific increase in Reelin expression. $A\beta_{1-42}$ also expanded SBM cellularity, with significant increases in neutrophils and monocytes, indicating an acute immune response. In 5xFAD mice, SBM niches were enlarged, and niche area and intensity correlated with Reelin expression. CR-50 treatment significantly reduced niche area and density in both AD models. MRI confirmed reduced SBM volume following treatment in APPNL-F mice. Conclusion: These findings identify SBM niches as active mediators of CNS–peripheral immune crosstalk in AD. Reelin serves as a potential marker of SBM activation, and its inhibition attenuates niche expansion, highlighting Reelin as a promising therapeutic target in AD-related neuroinflammation.

W186. B cell Depletion with anti-CD20 Promotes Mucosal-originating Regulatory IgA B cells in Multiple Sclerosis

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Abstract Text: Monoclonal anti-CD20 antibodies (anti-CD20) efficiently suppress inflammation and can reduce disability progression in multiple sclerosis (MS). They selectively deplete CD20-expressing B cells, while CD20-negative cells are spared. Anti-CD20 alters systemic levels of B cell–activating factor (BAFF) and a proliferation-inducing ligand (APRIL)—crucial factors for the survival and generation of regulatory IgA B cells. Yet, whether anti-CD20 and consequently altered B cell–regulating factors impact mucosal-derived regulatory IgA B cell responses in MS is unknown. Here, we performed longitudinal multi-omic profiling of blood, cerebrospinal fluid (CSF), and intestinal samples from anti-CD20–treated patients with MS. Findings were corroborated in a murine autoimmune encephalomyelitis (EAE) model. By applying an algorithm-guided analysis of high-dimensional spectral flow cytometry and single-cell RNA data, we found that anti-CD20 B cell depletion in MS is associated with increased

trafficking of regulatory, IL-10–producing IgA B and plasma cells from gut mucosal tissue into the systemic circulation and CSF. Furthermore, we observed persistently elevated systemic levels of BAFF and APRIL throughout the duration of anti-CD20 treatment, which were related to favorable outcomes in independent cohorts of MS patients receiving anti-CD20 therapy. Altogether, we identified novel regulatory effector mechanisms of anti-CD20–mediated B cell depletion with substantial implications for future therapeutic strategies that harness mucosal immune regulatory mechanisms in autoimmune diseases.

No Poster. Cytotoxic T Cell Recognition of α-synuclein Drives Pathogenic Immune Responses in Multiple System Atrophy

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Abstract Text: Multiple system atrophy (MSA) is a rapidly progressive and fatal α-synucleinopathy characterized by autonomic failure, parkinsonism, and cerebellar ataxia, for which no disease-modifying therapies currently exist. Neuroinflammation is a prominent feature of MSA pathology; however, the contribution of adaptive immune responses to disease pathogenesis remains poorly understood. In Parkinson's disease (PD), recent studies have demonstrated that T cells can recognize α-synuclein and acquire pathogenic effector functions, raising the possibility that similar immune mechanisms may operate in other synucleinopathies. Here, we comprehensively profile peripheral T cell responses in patients with MSA, in comparison with PD and healthy control (HC) cohorts, using single-cell transcriptomics, flow cytometry, and antigen-specific functional assays. We demonstrate that peripheral T cells from MSA patients exhibit strong activation and are skewed toward cytotoxic and inflammatory phenotypes. Single-cell RNA sequencing reveals clonal expansion of cytotoxic $CD8^+$ T cells expressing GZMB, GNLY, and transcriptional programs associated with chemokine- and integrin-mediated central nervous system (CNS) trafficking. Functionally, both $CD4^+$ and $CD8^+$ T cells from MSA patients recognize α-synuclein monomers and pre-formed fibrils in an HLA class I/II–dependent manner, driving proliferation, clonal expansion, and acquisition of cytotoxic features. Consistent with these peripheral immune responses, post-mortem analysis of MSA brain tissue shows increased $CD8^+$ T cell density in the parietal cortex compared to HC, including cytotoxic ($GZMB^+$, $GZMK^+$) and pro-inflammatory ($IFN\gamma^+$) $CD8^+$ T cell subsets, demonstrating that activated T cells are present at sites of neurodegeneration in MSA. Together, these findings identify α-synuclein-reactive cytotoxic T cells as an active component of MSA pathobiology, linking peripheral immune activation with CNS neuroinflammation and supporting adaptive immune pathways as a rational target for translational therapeutic strategies in MSA.

Reproductive Immunology

Tu184. The CXCL1/8-CXCR2 Axis Drives a Pathogenic Neutrophil-Macrophage Feedback Loop in Recurrent Pregnancy Loss

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Abstract Text: Objective: The disruption of maternal-fetal immune tolerance is a primary driver of recurrent pregnancy loss (RPL), mediated by a pathological inflammatory state in the decidua. As innate responders, neutrophils remain heavily understudied in this context due to their short half-life. Here, we aim to decipher neutrophil heterogeneity within the decidual microenvironment and elucidate its pathogenic role in RPL. Method: We performed a retrospective analysis of electronic health records from RPL patients and healthy controls (elective terminations) to evaluate systemic peripheral blood profiles. Concurrently, we integrated scRNA-seq and bulk RNA-seq deconvolution of human decidual tissues, employing an optimized alignment pipeline to resolve the neutrophil immune landscape. Findings were validated via immunofluorescence in clinical samples to confirm neutrophil expansion and explicit markers of NETosis. Mechanistic *in vivo* experiments were conducted in the CBA/J×DBA/2J RPL murine model utilizing a CXCR2 inhibitor, Cl-amidine, and DNase I. Parallel *in vitro* chemotaxis and co-culture assays evaluated CXCL1/8-mediated neutrophil recruitment, NET release, and macrophage polarization. Result: Clinical and transcriptomic analyses convergently revealed systemic and local neutrophil expansion in RPL patients. High-resolution subclustering resolved neutrophil heterogeneity into five distinct subsets, identifying an enriched, pathogenic $CXCR2^+$ subpopulation characterized by hyperactivated NETosis within the decidua. Receptor-ligand mapping established a conserved CXCL1/8-CXCR2 signaling axis between decidual M1 macrophages and $CXCR2^+$ neutrophils across human and murine models. Acting as the primary source of CXCL1/8, these macrophages actively drive $CXCR2^+$ neutrophil recruitment. *In vitro*, CXCL1/8 stimulation induced robust NETosis, with released NETs directly provoking M1 macrophage polarization. Crucially, *in vivo* CXCR2 blockade or targeted NET degradation abrogated M1 polarization, resolved decidual inflammation, and rescued fetal loss. Conclusion: We elucidate a novel, bidirectional crosstalk mechanism driving RPL. We demonstrate that abnormally secreted CXCL1/8 in the decidual microenvironment recruits a pathogenic $CXCR2^+$ neutrophil subpopulation. Upon recruitment, these neutrophils release neutrophil extracellular traps (NETs), which act as endogenous damage-associated molecular patterns (DAMPs) to provoke further pathological M1 macrophage polarization. This vicious inflammatory cascade breaks maternal-fetal immune tolerance. Ultimately, our findings highlight CXCR2-NET axis as a novel clinical biomarker and promising therapeutic target for RPL.

Tu185. Human Respiratory Syncytial Virus Causes Maternal Inflammation in Pregnant Mice and Behavioral Alterations in the Offspring

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Abstract Text: Human respiratory syncytial virus (hRSV) is one of the most prevalent respiratory viruses during the autumn and winter seasons. Classic symptoms of infection include cough, fever, and nasal congestion, which can progress to bronchiolitis or pneumonia in severe cases. The risk groups for this virus include children under 5 years of age, pregnant women, and older adults. hRSV mainly infects cells of the lower respiratory tract. In severe clinical cases of hRSV infection and in research conducted on cell lines, the virus can infect other cell types, including those

from the central nervous system (CNS) and placenta. The effects of hRSV infection during pregnancy on the offspring have not yet been studied. To address this, it is necessary to understand the changes this virus generates in the mother and fetus. This research aims to evaluate whether hRSV alters the behavior of offspring using a murine model. Pro-inflammatory cytokines and behavioral alterations were examined in mice born to mothers infected with hRSV. Increased levels of IL-6 and TNF-α were detected in the fetuses' brains. Moreover, the offspring of infected mice exhibited a behavioral phenotype associated with neurodevelopmental disorders. Our data support the hypothesis that hRSV infection during gestation causes maternal immune activation (MIA) and impairs the social behavior of the offspring.

W187. Maternal Immune Activation Triggers a Pathogenic "Senescence Chain" from Placenta to Offspring Brain Linking to Neurodevelopmental Disorders

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Abstract Text: Maternal immune status during pregnancy determines the lifelong risk of neurodevelopmental disorders (NDDs). Epidemiological evidence links prenatal pathogen exposure—including herpes simplex virus type 2, cytomegalovirus, and SARS-CoV-2—to maternal immune activation (MIA) and the etiology of autism spectrum disorder (ASD) and schizophrenia. While placental inflammation and disrupted cytokines (IL-6 and IL-17) mediate this risk, the transmission mechanisms to the fetal brain remain unclear. Here, we focused on cellular senescence, characterized by irreversible cell cycle arrest and the secretion of bioactive factors known as the senescence-associated secretory phenotype (SASP). While senescence plays a physiological role in healthy pregnancy, excessive senescence is linked to complications. We hypothesized that MIA triggers aberrant placental senescence, propagating to the offspring brain, leading to cytokine-induced neuronal senescence, neuroinflammation, and NDD-associated neural substrates. First, analysis of publicly available single-cell RNA-seq datasets from SARS-CoV-2-infected placentas revealed high senescence and SASP scores, with elevated IL-6 and IL-17 expression compared to uninfected controls. Next, using a poly(I:C)-induced MIA mouse model, we observed increased hippocampal senescence. Notably, the expression of the senescence marker Cdkn2a significantly correlated with behavioral abnormality scores. Gene expression analysis indicated a mixed M1/M2 microglial phenotype, and multiplex-IHC identified doublecortin-positive (DCX^+) immature neurons as the primary senescent population. To test whether the IL-6 and IL-17 observed in the placenta induce this neuronal senescence, treating HT-22 immature neuronal cells with recombinant cytokines revealed that while IL-6 alone induced senescence, the combination of IL-6 and IL-17 was required to induce potent SASP (IL-1α, IL-1β) and the "don't eat me" signal CD47. This CD47 upregulation and increased neurite length were confirmed in Doxorubicin-induced senescent cells and *in vivo* DCX^+ cells, suggesting aberrant synaptic formation. Finally, treating MG6 microglia with senescent HT-22-derived SASP upregulated SIRPA (CD47 ligand) and impaired phagocytic activity. Collectively, these results suggest a pathogenic "senescence chain axis": MIA-induced placental SASP triggers neuronal senescence in the offspring. These senescent neurons evade microglial surveillance via CD47 upregulation and suppress microglial function via SASP, leading to neuroinflammation and NDD-associated neural substrates.

W188. Unraveling the Immune Landscape of Chronic Placental Inflammation Across Gestation Through Multi-omics

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Abstract Text: The placenta is central to fetal development and long-term offspring health, and placental dysfunction is a major driver of adverse perinatal outcomes. Chronic placental inflammation (CPI) represents the most common placental pathology associated with late preterm birth and is strongly linked to unexplained fetal death and adverse developmental consequences. CPI is defined histologically by maternal mononuclear cell infiltration of placental tissues; however, the cellular composition, spatial organization, and molecular programs underlying CPI across gestation remain poorly understood. Here, we generated an integrated multi-omic atlas of CPI by profiling placental and extraplacental tissues from preterm and term pregnancies using imaging mass cytometry, single-cell RNA sequencing, spatial transcriptomics, T Cell receptor (TCR) repertoire and viral sequence mining, and genome-wide DNA methylation analysis. CPI emerged as a gestational age-dependent immune continuum rather than uniform inflammatory state, with distinct cellular architectures and signaling programs. Preterm CPI was characterized by diffuse immune infiltration dominated by cytotoxic $CD8^+$ T cells, activated macrophages, and natural killer cells within the extraplacental membranes, forming self-amplifying inflammatory circuits. In contrast, term CPI displayed spatially restricted immune niches enriched for homeostatic myeloid cells and regulatory T cells, suggesting active containment of inflammation and preservation of maternal-fetal tolerance despite ongoing immune activation. Multi-omic integration further revealed contributions from the developing fetal immune system in preterm CPI, including macrophages, T cells, and mast cells of fetal origin that reshaped maternal-fetal immune communication. Cell-cell communication analyses identified robust pro-inflammatory signaling networks in preterm CPI, whereas term CPI was associated with resolution-phase and regulatory pathways. TCR repertoire profiling identified expanded memory clonotypes with viral epitope similarity, consistent with antigen-agnostic or bystander activation. Finally, DNA methylation analyses revealed CPI-associated epigenetic remodeling linked to developmental and neurocognitive pathways in preterm birth. Together, this atlas defines the cellular, spatial, and molecular architecture of CPI across gestation and explains how chronic inflammation drives prematurity and offspring injury, or alternatively remains compatible with term delivery, providing a framework for translational biomarker discovery and therapeutic intervention development.

Stem Cell & Organ Transplantation

Th189. T cell Exhaustion After Kidney Transplant Is Associated with Thymoglobulin, Not Chronic Antigenic Stimulation

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Abstract Text: Background. T cell exhaustion after Kidney Transplant (KTx) is associated with increased infections and less rejection, but its etiology is unclear. The current study compared T cell markers including exhaustion from pretransplant to 2 years in older recipients receiving thymoglobulin (rATG) vs basiliximab induction. Methods. We retrospectively studied 42 cell surface markers by flow cytometry in paired PBMC samples (pretransplant and 2 years) in KTx recipients who were 54-74 years of age at KTx and had received either rATG induction (n = 13) or

basiliximab induction (n = 8). All were were CMV seropositive at KTx. At 2 years, lymphocyte counts were normal (≥950/μL) and none had rejection. Paired t-Test was used to compare changes. Results. The basiliximab group was older (66.2+5.2 vs 59.4+4.3 years) but otherwise not different (sex, type of transplant, race, or cause of renal failure). In the rATG group at 4 months, there were profound, widespread decrements in all T cell types. The rATG group (but not the basiliximab group) showed multiple changes at 2 years (Table) including: decreased number of $CD4^+$ T cells and their subsets (naïve, all memory subsets and Tscm, stem cell-like memory cells that maintain all memory subsets). $CD8^+$ T cell numbers and subsets were similar to baseline, except for naïve cells that decreased. At 2 years, the proportion of cells expressing exhaustion-related markers (PD-1 and/or TIGIT) was unchanged in the basiliximab group but increased in the rATG group including: overall $CD4^+$ T cells, $CD4^+$ T central memory, $CD4^+$ T terminal memory. Conclusions. T cell exhaustion after KTx is primarily due to rATG induction and not chronic antigenic stimulation (not seen in the basiliximab group) or rATG resistance (exhausted cells were depleted at 4 months). At 2 years, the decreased number of $CD4^+$ T cells combined with an increase in the proportion of exhausted $CD4^+$ T cells of all types suggests that the stress of immune reconstitution results in a unique form of T cell exhaustion that may affect long-term KTx outcomes. Cell type/t(t24m-t0m)/p-value $CD4^+$ -0.224/1.9991E-06 $CD4^+$TN -0.113/0.0002 $CD4^+$Tscm -0.014/0.008 $CD8^+$TN -0.034/0.006 $CD4^+$Exhausted 0.110/0.00003 $CD4^+$TCMExhausted 0.026/0.00098 $CD4^+$TTMExhausted 0.066/0.00049

Th190. Durable Leukemia-free and Severe GvHD-free Survival in Patients Treated with αβdepleted-HSCT and Tr1 Cell-enriched T-allo10 Immunotherapy

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Abstract Text: Allogeneic hematopoietic stem cell transplantation (HSCT) using $TCR\alpha\beta^+$ T Cell/$CD19^+$ B-cell-depleted (αβdepleted) grafts overcomes HLA mismatch barriers, but is limited by delayed immune reconstitution (IR) that increases the risk of viral reactivation and relapse. We hypothesized that infusion of T-allo10 cells, new donor-derived therapy enriched in patient alloantigen-specific type 1 regulatory T (Tr1) cells, will accelerate IR without increasing the risk of GvHD in αβdepleted allo-HSCT. Towards this, we conducted a phase Ia/Ib clinical trial (NCT04640987) in which 22 high-risk leukemia patients (AML, ALL, MDS, or MPAL; ages 1.7–24 years) received αβdepleted haploidentical HSCT, followed by three escalating doses of T-allo10 cells in the absence of post-transplant GvHD prophylaxis. Patients were evaluated for safety, IR (defined as ≥50 $CD3^+CD4^+$ T cells/μL by day +60), acute GvHD, relapse, and viral reactivations. Correlative studies included lymphocyte phenotyping by flow cytometry, high-dimensional plasma proteomics by Olink, *in vitro* antigen-specific response to viral peptides, and Tr1 tracking by flow cytometry and TCR sequencing. At one-year post-HSCT, we observed no dose-limiting toxicities and 0% non-relapse mortality. At the latest follow-up, patients treated with intermediate and high dose T-allo10 cells had 100% leukemia-free survival (LFS) and 100% of grade II GvHD- and -relapse-free survival (GRFS). Grade II acute GvHD occurred in five patients (23%), but all cases were steroid-responsive; there was no grade III-IV GvHD. From evaluable patients, 65% achieved IR by day +60 post-HSCT, doubling the rate observed in controls (patients treated with αβdepleted HSCT only). T-allo10 infusion rapidly increased memory $CD4^+$ T cells, led to durable Tr1 engraftment and reduction in viral reactivations at higher doses. *In vitro*, leukocytes from T-allo10 recipients produced more effector cytokines in response to adenovirus peptides than controls, while responses to cytomegalovirus peptides were maintained. Patients receiving intermediate and high T-allo10 doses had significant decrease in proinflammatory plasma proteins compared to controls. Altogether, infusion of therapeutic doses of Tr1-enriched T-allo10 cells post-αβdepleted HSCT is safe and highly effective, decreasing peripheral inflammation, accelerating immune recovery, and manifesting as sustained protection from relapse and severe GvHD. Thus, T-allo10 cells can transform the management of HSCT in pediatric and young adult patients.

Tu186. Resolving TCR-peptide Complexes for Donor-specific CD8⁺ Treg Induction and Graft Monitoring

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Abstract Text: Regulatory T cells (Tregs) are central to immune homeostasis, targeting them to induce graft tolerance is a promising alternative to conventional non-specific immunosuppression. Identifying donor-specific antigens recognized by Tregs could enable their selective expansion *in vivo* through a peptide-based vaccination and improve graft monitoring. Previous work in rat transplantation showed that donor MHC-II-derived peptides promote tolerance by inducing CD8⁺ Tregs. Our goal is now translate this strategy to humans. The Allele Frequency Net database was used to identify the most frequent HLA-I and II alleles in the European population. Clustal Omega enabled the design of peptide libraries from mismatched HLA-II sequences. NetMHCpan-4.1 and EasYmers (Immudex) were uses to predict and measure peptide affinity for HLA I protein. PBMCs from kidney transplant recipients (DIVAT biocollection, CHU Nantes) were analyzed to detect and characterize peptide-specific cells using spectral cytometry (Aurora, Cytek) combined with dextramers (Immudex) and with data processed in OMIQ. We identified 49 peptides with high HLA-I affinity, some of them shared a common motif (WO 2020/120786 A1). Peptide specific cells were detected in kidney transplanted patients with stable graft function, displaying a predominantly $CD45RC_{low}^{-}$ phenotype and a distinct signature. Some peptides triggered activation and clonal expansion of CD8⁺ Tregs, whose TCRs were associated with tolerance in public datasets. Together, these results will support the selection of candidate tolerogenic peptides and suggest a universal motif covering multiple mismatches.

Tu187. Enhanced T cell Suppression Is Associated with Impaired Donor-specific Treg Expansion and Absence of Sustained Donor-reactive T cell Clonal Deletion in Combined Kidney and Bone Marrow Transplantation

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Abstract Text: Combined kidney and bone marrow transplantation (CKBMT) with transient chimerism promoted allograft tolerance in a prior (ITN036) study in which three patients achieved tolerance, and one rejected their graft. Tolerance correlated with early post-transplant enrichment of circulating donor-specific Tregs (dsTregs) and decreases in donor-reactive T cell clones (DRTCCs). All patients developed chimerism transition syndrome (CTS) associated with transient renal dysfunction. To mitigate CTS in the current study, anti-CD2 was increased and an early course of mycophenolate mofetil (MMF) was introduced. We performed similar mechanistic analyses as described to assess their correlation with tolerance. To detect DRTCCs, unstimulated and donor-reactive (CFSElo) CD4 and CD8 T cells in pre-transplant MLRs were separately sorted by flow cytometry, DNA was extracted, and high-throughput CDR3 TCRβ sequencing was performed. To identify dsTreg clonotypes, irradiated stimulated donor B cells were cocultured with pre-transplant–sorted recipient $CD25^{+}CD127lo$ Tregs that were expanded for CDR3 TCRβ sequencing. Four of four patients exhibited transient chimerism, with reduced CTS compared to the prior cohort. None of the current patients achieved tolerance. Post-transplant DRTCCs did not show a consistent deletional pattern, instead exhibiting a relative increase in CD4 and/or CD8 DRTCCs 12–24 months post-transplant. Although early post-transplant Treg enrichment was observed, Treg expansion was non–donor-specific and transient, correlating with non-tolerance. These findings suggest that increased early T cell suppression reduced

CTS and prevented tolerance. The associated absence of DRTCC deletion or persistent dsTreg expansion suggests that these immunological outcomes may be required to achieve durable tolerance.

W189. Evidence for Immunomodulatory Effect of Thymus in Pig-to-decedent Human Thymokidney Transplant

Farshid Fathi[1], Pinar Gur Cetinkaya[1], Nathan Suek[1], Benjamin Vermette[1], Yasmeen Saad[1], Jeffrey Stern[2], Karen Khalil[2], Tal Eitan[2], Ian Jaffe[2], Imad Aljabban[2], Adam Griesemer[2], David Berglund[3], Idriz Limaj[4], Erik Berglund[4], Greg Nowak[1], David Sachs[1], Robert Montgomery[2] and Megan Sykes[1]

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Abstract Text: We aim to prevent xenograft rejection while avoiding long-term immunosuppression by inducing xenograft tolerance. Thymokidney (TK) transplants have achieved promising results in thymectomized baboons using only the GGTA1-KO genetic modification. Here, we describe a novel immunosuppressive regimen along with GGTA1-KO TK transplantation in a thymectomized human decedent recipient, characterizing T cell recovery over 22-days. TKs were constructed in an SLAhh GGTA1-KO miniature swine 6 months prior to transplantation of one TK into a decedent who underwent thymectomy and received immunosuppression consisting of siplizumab, obinutuzumab, IgG degrader, belatacept, eculizumab, pegcetaloplan and steroids. The experiment continued for 22 days and immune cell subsets in the contralateral non-transplanted TK, decedent's peripheral blood before and after transplantation, and explanted TK graft were characterized by FCM. scRNA and TCR-seq were performed on explanted thymokidney. Renal function was excellent throughout the experiment (Cr < 1.0 mg/dl). Normal pig thymopoiesis was evident contralateral non-transplanted TK (70% double positive (DP)). Single positive (SP) CD4 cells in the thymus included 33.8% pig Tregs. These populations were also detected in the kidney beneath the thymus (DP 37%, Treg 17.5%), while kidney sections further from the thymus contained fewer thymocytes (DP 1.4%). In the decedent, T cells in peripheral blood began to recover by POD14, peaking on POD22 (115 cells/μL), including high proportions of $CD4^+$ recent thymic emigrants (43% on POD21). The thymic portion of the explanted TK on POD22 contained pig and human $CD45^+$ cells (2.79% pig $CD45^+$, 36.5% human $CD45^+$), including DN, DP, SPCD4, SPCD8 and Treg cells. Pig Tregs comprised 54.8% of pig SPCD4s and human Tregs comprised 5.74% of human SPCD4s. The kidney region below the thymus also contained pig and human T cells, including high proportions of pig (78% of SPCD4s) and human Tregs (9.27% of SPCD4s). scRNA seq data showed enrichment of Tregs (21%). Human thymocytes and T cells, including Tregs, were detected within the thymic tissue at explant, and human RTEs were recovered in peripheral blood. The presence of porcine Tregs suggests a possible "immunomodulation" mechanism that may explain the outstanding outcomes achieved in pig-to-baboon TK xenografts using source pig with only the GalT-KO modification

W190. Airway CXCL13 Identifies a B cell-prominent Endotype of Chronic Lung Allograft Rejection Which May Guide Early Rituximab Intervention

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Abstract Text: Although B cells and tertiary lymphoid structures (TLS) are key drivers of allograft rejection, the efficacy of B cell-depleting therapy, rituximab (RTX), in solid organ transplantation remains limited. RTX is often administered too late—only after donor-specific antibodies (DSAs) are detected during late stages of disease. Further, whether RTX can adequately penetrate tissue to target B cells within lung TLS remains unclear. To

investigate whether a B cell-prominent endotype of rejection can be identified, we used bulk-RNAseq to characterize 44 chronically rejected human lung allografts and 27 healthy lung explants. CXCL13, encoding a B cell-recruiting chemokine, was highly differentially expressed in a subset of rejected allografts and associated with a broader B cell-related gene signature (CD19, CCL3, CR2, FDCSP, TNFRSF13C, BLK, CD79A, MS4A1, PAX5). Allografts with a higher representation of this B cell gene signature displayed greater TLS-formation and fibrosis on histology. In this same patient cohort, we performed a 96-plex protein analysis on broncho-alveolar lavage fluid collected from the airways at the time of lung function decline to assess the presence of various immune mediators, including other B cell-related cytokines. We found that CXCL13 was the top protein candidate associated with allograft fibrosis, as assessed by chest imaging, and with reduced patient survival. In an independent validation cohort of 295 lung transplant recipients, we found that CXCL13 was detectable in the airways as early as 6 months post-transplant and was associated with increased risk of DSAs, chronic rejection, and re-transplantation/death. In a mouse model of chronic lung allograft rejection, late RTX treatment—initiated after TLS formation and antibody production, consistent with current clinical practice—effectively depletes B cells in the circulation but fails to completely eliminate B cells residing in the allograft, lymph nodes, and spleen, as assessed by flow cytometry with *in vivo* intravascular leukocyte labeling. By contrast, RTX administered early post-transplant, at the time of initial CXCL13 release, prevents B cell extravasation and subsequent TLS formation within the allograft. Accordingly, clinical detection of a B cell-prominent endotype of chronic rejection, as indicated by airway CXCL13 levels, may facilitate patient risk-stratification and timely initiation of B cell-targeted therapies.

Systems Immunology

Th149. Auto-immunity Risk Assessment: Use Cases of Molecular Mimicry Hypothesis for Viral Infections and Therapy Designs for Vaccines-associated Adverse Events

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Pfizer

Abstract Text: Autoimmunity refers to an aberration in the body's normal development that causes the immune system to mount an attack against its cells. The etiology behind autoimmune diseases is multifactorial, with genetic, hormonal, and environmental factors all playing a role. One theory of how autoimmune diseases develop is through the process of molecular mimicry (MM), where the immune system is exposed to a foreign molecule that is similar to a self-molecule, so that antibodies can cross-react with the self-molecule and trigger an immune response against the body's own tissues. MM has been proposed as a potential mechanism underlying several autoimmune diseases post infections by SARS-CoV-2, Epstein-Barr Virus and Group-A Streptococci, including myocarditis, multiple sclerosis, and rheumatoid arthritis. This poses a great challenge in understanding complications after infections for some individuals and designing vaccines against these pathogens, as autoimmunity is a potential safety concern. This study focuses on building a computational strategy to derisk adverse effects due to MM. For T Cell mediated MM, we used blast algorithms to identify pathogenic peptides/regions that are similar enough to human peptides and then immune epitope database (IEDB) for epitope predictions. For B-cell mediated MM, we used AlphaFold to identify pathogenic surface regions that are similar enough to surface regions of human proteins. With help of omics data, the identified human proteins were further prioritized, depending on the tissue of interest (e.g. heart for myocarditis). Infectious disease, vaccine and safety scientists can highly benefit from our computational approach for hypothesis generation and safety risk assessment.

Th191. Development of a Novel Ultrasensitive Multiplex Proteomic Panel for Comprehensive Profiling of Immune and Inflammatory Biomarkers

Xiao-Jun Ma, Haiyan Wu, Roopa Comandur, Victoria Torres, Joanne Beer, Kavita Tirumale, Sean Kim, HaYeun Ji, Niyati Jhaveri, Xiaolei Qiu, Bingqing Zhang and Yuling Luo

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Abstract Text: Comprehensive profiling of biomarkers linked to inflammatory, autoimmune, and infectious diseases is critical for advancing diagnostic and therapeutic development. Progress in this area has been constrained by the lack of highly sensitive multiplex platforms that can detect across the large dynamic range of protein concentrations. To address this gap, we developed NULISAseq Immune 340 Panel, enabling simultaneous quantification of over 340 proteins involved in inflammation and immunology. The panel provides comprehensive coverage of critical pathways, including apoptosis, immune checkpoints, complement activation, and key disease-associated markers relevant to systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), sepsis, and cancer. This high multiplex assay was optimized for plasma and serum and underwent extensive analytical characterization to assess precision, reproducibility, detectability, specificity, and dilution linearity. Results demonstrated strong analytical robustness, with median intra- and inter-plate CV below 10%. Over 90% of targets exhibited excellent dilutional linearity (RSQ >0.9) and >50% detectability in plasma samples with high signal-to-noise ratios, while cross-reactivity remained under 1%, confirming high specificity. Following validation, the panel was applied to a heterogeneous disease cohort, revealing distinct, condition-specific biomarker signatures that reflect underlying immunopathology. These findings underscore the potential of this platform to uncover novel disease mechanisms and enable patient stratification based on molecular profiles. In summary, the NULISAseq Immune 340 Panel represents significant advancement in immune biomarker analysis, combining breadth, sensitivity, and reproducibility in a single assay. By enabling deep characterization of immune pathways across diverse conditions, it opens new opportunities for early disease detection and longitudinal monitoring in biofluids.

Th192. Spatiotemporal Coordination of Immune and Stromal Responses in Human Skin Following Inflammatory Perturbation

Christine Dien[1], Jacob Kim[1], Andrew Johnston[1], Ao Huang[1], Meirav Sela[1], Margaret MacGibeney[1], Can Liu[1], Shoham Benmelech[1], Yona Lei[1], SiYi Chen[1], Leon Bichmann[1], Anjali Jaiswal[1], Michal Kidacki[1], Matthew Vesely[1], Surender Khurana[2], Hana Golding[2], Andrew Martins[1], Inci Yilidirim[1], Rachel Sparks[1] and John Tsang[1]

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Abstract Text: The skin plays a critical role in protecting against environmental and pathogenic insults. It comprises an intricate organization of stromal and immune cells maintaining homeostasis and responding to perturbations. As a natural barrier, the skin is a site of frequent inflammation involving immune cell trafficking through its robust lymphovascular network. Key functions of cutaneous stromal cells are their roles as signal amplifiers and recruiters of immune cells, yet the spatiotemporal dynamics of these interactions in human tissue remain poorly understood. To characterize both temporal and spatial organization of skin-mediated immune responses, we employed intradermal influenza vaccination as a controlled perturbation model. We longitudinally collected skin biopsies from healthy volunteers (n=38) before vaccination (baseline/Day 0) and at multiple post-vaccination timepoints in separate groups of participants (2 hours, 6 hours, and Days 1, 3, 28). Using 10x Xenium spatial transcriptomic to profile these samples, we captured immune and stromal dynamics with joint temporal and spatial resolution. We observed signals of inflammatory responses in several stromal and immune cell types, primarily characterized by TNF-α and IFN-associated inflammatory programs peaking around 6 hours and 1 Day post-vaccination, respectively. Notably, we observed pronounced cell-state remodeling of stromal cell types, including fibroblasts, endothelial cells, and keratinocytes, with some cell-state changes retained until Day 3 (keratinocytes) and Day 28 (fibroblasts and endothelial cells). Access to the spatial locations of cells allowed us to identify gene sets that vary as a function of dermal depth and proximity to blood vessels, with some programs displaying coordinated spatiotemporal regulation.

These patterns highlight structural constraints on tissue immune dynamics and immune cell trafficking, underscoring an active role for stromal cells in shaping immune activation, migration, and resolution. Together, our findings demonstrate coordinated spatially and temporally organized interactions between immune and stromal cells during human cutaneous inflammation and establish intradermal vaccination as a powerful model for studying tissue-level immune trafficking and response dynamics in humans.

Th193. Baseline Signatures Reveal Shared Axes of Immune Activation and Response Across Contexts

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Abstract Text: Systems immunology studies of blood transcriptomic changes in response to vaccination, infection, aging, and immune disorders have identified numerous baseline gene set signatures predictive of immune function and dysfunction. The extent to which these signatures capture shared immune processes and pathways is unclear, given that there is little overlap between gene sets and functional insights from gene set enrichment analyses are fairly limited. Previous work from our group has demonstrated that baseline immune variation can correspond to differential priming of immune pathways resulting in varying acute response capacities across individuals and populations. Based on this observation, we hypothesized that the various baseline gene set signatures uncovered in diverse cohorts and immune contexts capture shared pathways involved in immune activation and response. Calculating various baseline gene set signature scores for a clinically healthy human cohort, we find that despite few shared genes, these signatures encode shared biological information and capture immune variation along two main axes. Immunologically, these axes together correspond to (a) interferon response and signaling, (b) neutrophil activity, (c) lymphoid cell activation, and (d) cytotoxicity. Analyzing the immune response to multiple vaccines across diverse cohorts, we find that acute (one day after vaccination) changes in blood transcriptomic profiles are along the same two axes. Samples collected during early infection response also showed changes along the same axes. The two axes were significantly different between clinically healthy individuals and those with monogenic immune disorders, systemic lupus erythematosus, or juvenile dermatomyositis. There was little variation along these axes with age. Altogether, our analysis links baseline gene set signatures identified in heterogeneous contexts to shared axes of immune variation at baseline, during acute response to vaccines and infection, and in multiple immune disorders, highlighting shared regulatory mechanisms across diverse immune contexts.

Tu188. Serum Biotin Concentrations in the Korean General Population: Implications for Biotin Interference in Immunoassays

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Abstract Text: Background: The use of biotin supplements for hair, skin, and nail health is increasing, raising concerns about interference in immunoassays that rely on the biotin–streptavidin interaction. This study investigated the distribution of serum biotin concentrations in the Korean general population to estimate the potential risk of biotin interference. Methods: A total of 400 serum samples were collected from individuals aged 18–79 years who underwent routine health checkups between September 2024 and March 2025. Serum biotin concentrations were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Possible biotin interference in triiodothyronine (T3), free thyroxine (free T4), thyroid-stimulating hormone (TSH), troponin T, and vitamin D immunoassays was evaluated in samples with biotin concentrations exceeding 10 ng/mL. Results obtained using a streptavidin–biotin-based electrochemiluminescent immunoassay (ECLIA) were compared with those obtained using chemiluminescent microparticle immunoassays (CMIA) or LC-MS/MS, as appropriate. Results: Serum biotin concentrations above the lower threshold for potential biotin interference (10 ng/mL) were observed in 0.5% (2/400) of samples. In one participant with a serum biotin concentration of 140.1 ng/mL, the vitamin D level was within the

normal range by ECLIA (31.7 ng/mL), whereas it was classified as deficient by CMIA (28.4 ng/mL) and LC-MS/MS (28.6 ng/mL). Conclusions: Based on the observed distribution of serum biotin concentrations, the overall risk of biotin interference in this population appears to be low. However, given the increasing use of biotin supplements, continued monitoring and further studies are warranted.

Tu190. A 61-Parameter CyTOF Panel for Comprehensive Profiling of Human PBMC to Characterize Immune Activation, Checkpoint Expression and Cytokine Signatures

Stephen Li, Michael Cohen, Katrina Thomson, Lauren Tracey and Christina Loh

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Abstract Text: High-parameter cytometric analysis enables discoveries of potential therapeutic targets and informs disease prognoses in translational and clinical research. CyTOF™ technology allows for the simultaneous measurement of over 50 markers in a single tube without the need for spectral unmixing or single-stain controls. This enables rapid design and modification of high-parameter panels to assess lineage, activation, exhaustion and intracellular signaling pathways in parallel. In this study, we developed a 61-parameter CyTOF panel optimized for functional immune profiling to support cancer immunology research. The panel includes markers for major immune subsets and T cell phenotyping targets, covering activation, differentiation, checkpoints (for example, PD-1, CTLA-4, TIGIT) and cytokines relevant to tumor immunity. PBMC were barcoded, pooled and stained, then cryopreserved and acquired on a CyTOF XT PRO system to minimize technical variability. High-dimensional analysis of stimulated PBMC demonstrated the panel's ability to resolve diverse immuno-functional states at the single-cell level. Co-expression patterns of activation markers, immune checkpoints and cytokines were observed across effector, memory, cytotoxic, regulatory and exhausted subsets. These findings underscore the panel's potential to identify immune signatures associated with tumor immunity and immunotherapy response when applied to patient samples or tumor-associated immune cells. CyTOF systems enable the highest number of parameters in a single panel, supporting comprehensive immune profiling and detailed interrogation of intracellular targets. Overall, this approach provides a powerful tool for studying functional immunology in cancer, offering insights into mechanisms that inform clinical decision-making and therapeutic strategies. For Research Use Only. Not for use in diagnostic procedures.

Tu191. Strong False-positive Pre-transplant Flow Cytometric Crossmatch Induced by Non-HLA Autoantibodies in a Non-Sensitized Renal Transplant Candidate

In Hwa Jeong, Bo Young Yoon, Ae Jung Kang and Young Soo Chung

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Abstract Text: Background: Pretransplant flow cytometric crossmatch (FCXM) testing is a cornerstone of kidney transplantation work-up, as a positive T Cell FCXM typically indicates the presence of donor-specific anti-HLA class I antibodies and an increased risk of antibody-mediated rejection (AMR). However, discordant results may occur when T Cell FCXM is strongly positive despite persistently negative HLA antibody screening by single antigen bead assay (SABA), creating significant diagnostic and clinlical uncertainty.In such cases, non-HLA antibodies, particularly autoantibodies, have emerged as a potential explanation for false-positive FCXM results. Case presentation: We report a 46-year-old non-sensitized male with end-stage renal disease on maintenance hemodialysis who underwent deceased donor kidney transplantation work-up. The patient had no prior history of transfusion, transplantation, or other sensitizing events and showed consistently negative HLA class I and II antibodies on serial SABA testing over several years. During pretransplant evaluation, complement-dependent cytotoxicity crossmatch was negative for both T and B cells, while FCXM revealed a strong isolated T Cell positivity. Extensive ancillary testing, including serial serum dilution, heat treatment, and dithiothreitol treatment, excluded prozone effect and clinically significant HLA donor-specific antibodies. Subsequent non-HLA antibody screening identified multiple autoantibodies, including those directed against FLRT2, vimentin, tubulin alpha-1B chain, GAPDH, protein kinase C zeta, CD36, and collagen

III. These findings were considered the most plausible cause of the discordant T Cell FCXM positivity. Discussion and Conclusion: This case highlights the diagnostic complexity of interpreting isolated T Cell FCXM positivity in the absence of HLA donor-specific antibodies. Although non-HLA antibodies have been implicated in graft injury and rejection, their pathogenic relevance in the pretransplant setting remains incompletely defined, and current evidence does not support their use as absolute contraindications to transplantation. Our findings emphasize the importance of considering non-HLA autoantibodies in cases of unexplained FCXM positivity to avoid unnecessary exclusion of transplant opportunities. Systematic evaluation of non-HLA antibodies may aid in distinguishing HLA-mediated alloimmunity from non-HLA-mediated autoimmunity, enabling more informed clinical decision-making and individualized transplant planning.

W191. Longitudinal Single-cell Multi-omics Profiling of Human Household and Vaccination Cohorts Reveals Perturbation-linduced Baseline Immune State Changes

Yona Lei[1], Leon Bichmann[1], Amy Callear[2], Sarah Cobey[3], Benjamin Cowling[4], Faith Ho[4], Can Liu[1], Emily Martin[2], Andrew Martins[1] and John Tsang[1]

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Abstract Text: The immune system exhibits substantial intra- and inter-individual variability, posing a major challenge for developing interventions that are effective across diverse populations. Underlying this heterogeneity are personal baseline immune states, or immune setpoints, that arise from the complex interplay of intrinsic factors and cumulative exposures. However, it remains unclear how common immune challenges such as viral infection and seasonal vaccination modulate immune setpoints, and which baseline features are informative predictors of functional outcomes across individuals and contexts. We address this gap by integrating multimodal analyses of longitudinal peripheral blood mononuclear cell (PBMC) samples from two human cohorts. In a cross-sectional study, we previously showed that healthy males recovered from mild COVID-19 infection exhibited a more poised baseline state linked to better responses to subsequent influenza vaccination. To determine the impact of natural infection on antigen-agnostic baseline immune states while controlling for shared environmental exposures, we analyzed same-season PBMC samples from age-matched individuals from the same household using single cell profiling of surface proteins, transcriptomics, TCR/BCR, and chromatin accessibility. Using influenza infection as a model, we found distinct, durable innate immune alterations in recovered individuals months after PCR-confirmed infection. We also identified potential baseline predictors of protection in uninfected household counterparts, including elevated inflammatory signatures in innate immune cells and hematopoietic stem and progenitor cells at the start of the flu season. To broadly assess the molecular and cellular changes induced by vaccination, we similarly profiled participants from a four-year randomized controlled influenza vaccination trial, comparing first-time vaccinees to repeat vaccinees who were vaccinated annually. We found that repeat vaccinees were characterized by a more dampened immune signaling and activation profile at baseline. Moreover, repeat vaccinees demonstrated the typical resolution of vaccination-induced inflammatory responses by day 30 post-vaccination, whereas first-time vaccinees maintained persistent immune activation signatures. Consistent with this heightened baseline state, innate immune cells from first-time vaccinees demonstrated greater response capacity to *in vitro* LPS stimulation. Our findings show that common immune perturbations can modulate immune setpoints with potentially long-lasting functional consequences, providing a framework for predicting individual immune trajectories and identifying targets to modulate immune responses and health outcomes.

W192. A Comprehensive Catalog of B cell Expression Programs Reveals New Disease-associated Functional States

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Abstract Text: Therapeutic deep B cell depletion has yielded durable drug-free remissions for multiple autoimmune diseases. However, the specific pathogenic populations explaining these outcomes remains unclear. Better characterization of B cell subsets could enable targeted rather than complete B cell depletion. We previously described star-CellAnnoTator (starCAT), a pipeline for discovering reproducible gene expression programs (GEPs) across multiple datasets (Kotliar Et. Al, 2025). Applying starCAT to T cells improved characterization of polarization, cytotoxicity, and TCR-activation states. Here, we apply starCAT to ~1.25 million B lineage cells from over 2000 samples across 49 tissues in healthy contexts, cancer, COVID-19, and rheumatoid arthritis. Integrating across datasets yielded 19 consensus GEPs (cGEPs) associated with lineage (e.g., naive, transitional, non-class switched memory, and age-associated B cell) and 24 associated with activity states. Examining cGEPs following experimental BCR stimulation shed light on the cellular rewiring induced by activation and enabled identification of activated cells in disease datasets. The most strongly BCR-activation associated cGEP, NME1/FABP5 includes glycolytic flux regulators and was previously identified following T cell activation, implicating it as a general lymphocyte activation metabolic response. Another BCR-activation cGEP, EBI3/CXCL9, shows the strongest association with COVID-19 infection aside from interferon response and reflects a chemoregulatory activation response. Finally, because starCAT uses pre-defined cGEPs, it overcomes sparsity in spatial transcriptomic data to improve cell state annotation. Analyzing Xenium data from breast adenocarcinoma, we identify spatial co-localization of activated T cells and age-associated B cells. Overall, our B cell annotation strategy enhances characterization of pathogenic B cells within genomic datasets.

W193. Spatiotemporal Mapping of the Intestinal Axis Reveals Immune–epithelial Programs Driving Mucosal Inflammation and Recovery

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Abstract Text: The intestinal mucosa exhibits profound regional specialization that shapes host defense against enteric pathogens. However, the coordination of immune and epithelial cell programs across the ileum and colon during homeostasis and infection remains incompletely understood. Citrobacter rodentium (Cr) infection of the mid-distal colon models key features of inflammatory bowel disease and attaching/effacing bacterial infections, but existing single-cell studies have examined isolated intestinal regions or lacked spatial resolution. Here, we generated a single-cell atlas spanning the ileum, proximal, middle, and distal colon from C57BL/6J mice, comprising over 650,000 cells at baseline, during acute infection (day 8), and early resolution (day 14), resolved into 103 cell states. In parallel, we generated a matched Xenium spatial transcriptomics atlas for direct spatial mapping of transcriptional programs. This resource revealed two novel goblet cell states: a precursor-like state enriched during acute infection (GC precursor Cr–induced) and a pathogen-induced goblet cell state emerging during recovery (GC Cr–induced), both spatially linked to bacterial load and tissue pathology, suggesting distinct roles in pathogen response and tissue regeneration. To dissect multicellular communication underlying these region-specific responses, we performed integrative analysis using LIANA+, identifying temporally and spatially restricted ligand-receptor networks organizing communication across epithelial, myeloid, and lymphoid neighborhoods during infection. Regulatory network inference via pySCENIC uncovered infection-induced transcription factor programs distinguishing pathogenic from homeostatic responses, while cNMF revealed coordinated inflammatory gene modules spanning multiple cell types. Metabolic modeling using scCellfie identified epithelial cell-state-specific metabolic rewiring associated with antimicrobial defense and tissue repair. Preliminary spatial analysis maps these inflammatory and repair programs to distinct tissue niches across infection stages, with evidence enabling spatial mapping of cNMF-defined inflammatory programs and supporting predicted ligand-receptor interactions through cellular proximity. Together, this

spatiotemporally resolved systems atlas of the intestinal axis during Cr infection uncovers immune-epithelial programs dynamically orchestrating mucosal inflammation, repair, and recovery.

W194. Characterization of Peripheral Immune Variation During Immunotherapy Response and Toxicity Using High-resolution Immune Profiling and Machine Learning

Duncan McKenzie[1], Carla Castignani[1], Jack Bibby[1], Max Emmerich[2], Jeremy Mason[1], Jennie Yang[1], Marija Miletic[2], Laura Marandino[2], Zayd Tippu[2], Jonathan Lim[2], Taja Barber[2], Stephanie Hepworth[2], Paul Rouse[1], Lilian Williams[1], Kim Edmonds[2], Justine Korteweg[2], Laura Boddy[2], James Larkin[2], Tom Hayday[1], Samra Turajlić[2] and Adam Laing[1]

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Abstract Text: The immune system is a central determinant of health and disease, but its complexity and plasticity pose a challenge for comprehensive assessment. To realize the potential of complex immune analysis for diagnostic and precision medicine, it is essential to overcome existing challenges in assay and analytical standardization and throughput. Our novel machine learning (ML) platform enables high throughput real-time immune cell classification, facilitating standardized, robust and reproducible characterization of over 2000 immune cell subsets, states and phenotypes. We profiled 719 longitudinal blood samples from 47 renal and 86 skin cancer patients enrolled in the EXACT study with high-resolution spectral flow cytometry spanning 60^{+} markers and contextualized them alongside IMU Bioscience's reference set of 1000^{+} healthy individuals. A subset of samples was also profiled with single cell RNA sequencing to provide additional mechanistic context. At the cohort level, cancer samples diverged significantly from healthy donors, revealing distinct global disease- and treatment-associated alterations to immune architecture. Latent representations of healthy immune structure further allowed quantification of individual-level deviations from the reference in cancer samples. Initiation of immune checkpoint blockade therapy drove global shifts in immunophenotype that varied in magnitude and duration between patients over the course of treatment and follow up. In addition, IMU's direct profiling of immune checkpoint inhibitor target-bound immune cells enabled dynamic monitoring of treatment response throughout the disease course. Given the depth of longitudinal sampling, we further characterized latent trajectories of immune-related adverse event development, yielding mechanistic insights into toxicity. These findings were elaborated using single cell transcriptomics, highlighting the complementarity of these profiling approaches. 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 in cancer.

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