ILC6 2026 Abstracts:

Oral Presentations- Alphabetical by Submitter

Asif, Sara: University of Ottawa.

- *ILC2s in Ovarian Health and Disease*

Group 2 innate lymphoid cells (ILC2s) function as first responders to physiological tissue inflammation and, during malignant transformation, can direct tumor immunity in its early stages. The ovary represents a unique interface where tissue inflammation is necessary to support ovarian function, such as ovulation. However, with age, repeated cycles of ovulation alongside sustained inflammation can introduce a fibrotic niche within the ovary, possibly making it more susceptible to developing cancer.

Here, we identified ILC2s in the mouse ovary and found that they represent a dynamic population regulated by hormonal signaling and estrus cycling. ILC2 abundance predominated during the pre-ovulatory stage of the estrus cycle and significantly decreased post-ovulation. In parallel, IL-33, a key cytokine that stimulates ILC2s, was abundantly expressed pre-ovualation, and blocking the IL-33 receptor significantly reduced ovulation efficiency.

Having identified ILC2s in the healthy mouse ovary, we next sought to extend our studies to ovarian cancer. Using an orthotopic mouse model of ovarian cancer, we found that IL-33 treatment increased ILC2 abundance, activation, and effector function. Furthermore, IL-33-activated ILC2s upregulated surface expression of PD-1, suggesting the potential for combination therapy with anti-PD-1 immunotherapy. Indeed, IL-33 + aPD-1 treatment significantly improved survival in mice bearing ovarian tumors. IL-33-activated ILC2s also expressed high levels of the effector cytokines IL-5 and IL-13. ILC2-derived IL-5 enhanced B1 cell abundance in tumors, draining lymph nodes, and peritoneal lavage of tumor-bearing mice. Lastly, B cell depletion abolished the survival benefit of IL-33 + aPD-1 treatment. These findings uncover an ILC2–B cell axis in which IL-5 plays a critical role in driving B1 cell–mediated humoral immunity to promote antitumor responses in ovarian cancer. Collectively, our findings reveal that ILC2s are present in the normal ovary, where they respond to tissue inflammation, and in ovarian tumors, where they help direct anti-tumor immune responses.

Bernink, Jochem: Amsterdam University Medical Center.

- *Airway tuft cells govern homeostatic and infection-induced innate lymphoid cell reprogramming*

The human airway epithelium is continuously exposed to diverse microbial and environmental challenges, and relies on rapid and appropriate calibrated protective immune responses.

The heterogeneous family of innate lymphoid cells (ILCs) reside in close proximity to the airway epithelium, where their functional diversity enables prompt responses to a wide range of stimuli. However, how ILC heterogeneity is maintained and restored in a predominantly type 2-oriented environment, remains unclear. Using primary human ILCs and airway organoid culture systems, we report on a layered epithelial-immune circuit that preserves and dynamically reprograms ILC heterogeneity. At steady-state, human ILC2-derived IL-13 drives differentiation of pulmonary tuft cells, a subset of which constitutively secretes IL-23 to support ILC3 maintenance. Infection with the common pathogen Pseudomonas aeruginosa disrupts this homeostatic state by inducing IL-23 expression across multiple epithelial cell types in a TLR5-dependent manner. Concomitant IL-1b production by tuft cells, upregulates IL-23R on ILC2s, rendering them responsive to IL-23 and redirecting them toward protective IL-17A production, while maintaining IL-13 expression. Pre-existing ILC3s mount a robust IL-22 response, which together with the ILC2-derived IL-17A acts on epithelial cells to induce mucus production, antimicrobial peptide and chemokine expression. Taken together, these findings reveal a dual role for airway epithelial cells in maintaining homeostatic type 2-to-type 3 crosstalk, and in rapidly coordinating protective innate immunity during bacterial infections

Boulenouar, Selma: Harvard University.

- *Microbiota-Metabolic Crosstalk Drives Selective Intestinal ILC3 Loss*

Group 3 innate lymphoid cells (ILC3s) are critical sensory hubs that sustain intestinal immunity while adapting to environmental cues such as food intake and microbiota composition. Nutritional stress, particularly high-fat diet (HFD) feeding, disrupts ILC3 homeostasis, but the mechanisms underlying this disruption remain elusive.

Here, we show that intestinal ILC3s are markedly reduced in overweight and obese humans, paralleling findings in HFD-induced obese mice. Surprisingly, this loss is independent of caloric excess, weight gain, or glucose intolerance. Instead, ILC3 impairment begins within hours of HFD consumption, coinciding with microbial efflux that reprograms tissue-resident mononuclear phagocytes (MNPs) toward a proinflammatory phenotype, thereby driving ILC3 cell death.

Mechanistically, we demonstrate that microbiota-derived stimulation overrides the ability of lipid-laden ILC3s to engage fatty acid oxidation (FAO). This "effector–FAO" antagonism results in the accumulation of lipid peroxides and subsequent mitochondrial dysfunction, ultimately compromising ILC3 survival. Importantly, Th17 cells remain unaffected by this metabolic conflict, revealing a distinct immunometabolic signature unique to ILC3s that is conserved in both mice and humans.

Collectively, these findings uncover a previously unrecognized microbiota–lipid crosstalk that selectively impairs intestinal ILC3s, while sparing Th17 and other ILC subsets. By defining this

lineage-specific vulnerability, our work opens new avenues to target ILC3s in regulating intestinal homeostasis and inflammation, with potential implications for metabolic and inflammatory disorders associated with dietary stress.

Burrows, Kyle: Simon Fraser University.

- *cMAF-dependent NCR+ecto-ILC3 govern the inflammatory magnitude of inflammatory bowel disease.*

Group 3 innate lymphoid cells (ILC3s) are a heterogenous population of cells that regulate intestinal immune homeostasis and inflammation through the production of cytokines that act on epithelial and myeloid cells. However, the extent to which functional specialization within the ILC3 pool contributes to the intestinal inflammatory response remains poorly define.

Here, we identify a unique subset of NCR^{+}ILC3 cells that co-express the ectonucleotidases CD39 and CD73. These NCR^{+}ecto-ILC3 arise from NCR-ILC3, upregulate NCRs and ectonucleotidases, express high levels of the transcription factor cMAF and differentiate upon stimulation with interleukin(IL)-23. Targeted deletion of cMaf in NCR+ cells fully abrogated the development NCR+ ecto-ILC3s resulting in impaired production of inflammatory cytokines and chemokines. Animals lacking NCR+ecto-ILC3s showed decreased numbers of tissue-resident myeloid cells. In line with an impaired production of inflammatory cytokines in NCR+ecto-ILC3 deficient animals, anti-CD40 driven inflammatory bowel disease showed attenuated pathology and improved disease outcome when NCR+ ecto-ILC3 development was blocked.

These findings demonstrate that c-MAF-dependent, IL-23 responsive NCR+ecto-ILC3 are a previously underappreciated ILC3 subset that controls the severity of inflammatory bowel disease by governing the inflammatory magnitude.

Chen, Nan: University of Toronto.

- *An intestinal IL-33-ILC2 axis regulates sexual dimorphism in diet-induced obesity-related insulin resistance*

Obesity-associated insulin resistance (IR) is more severe in males than in females, but the mechanisms underlying this sex bias remain unclear. Using single-cell RNA sequencing, flow cytometry, and in vivo mouse model validations, we identify a fibroblast-derived IL-33–ILC2–barrier axis in the colon that is selectively compromised in obese males. High-fat diet (HFD)–fed males show loss of the colonic group 2 innate lymphoid cells (ILC2) and $IL\text{-}33^{+}$ fibroblasts, defective tight junctions and mucus, and worsened IR, whereas females are relatively protected from these changes. Hematopoietic Rorα deficiency and Il33 deletion exacerbate metabolic dysfunction and intestinal barrier failure, especially in females, while ILC2 adoptive transfer or recombinant IL-33 restore ILC2 abundance, barrier integrity, and insulin sensitivity in males. Androgen and IFNγ collectively inhibit ILC2 and $IL\text{-}33^{+}$ fibroblasts, whereas castration and IFNγ

neutralization expand IL-33^{+} fibroblasts and ILC2 to improve metabolic control. Pharmacologic RORα activation with nobiletin enhances colonic ILC2, strengthens the intestinal barrier, and ameliorates IR in a RORα-dependent, male-biased manner. Together, these data define a putative fibroblast-ILC2-barrier circuit that underlies sex-biased susceptibility to obesity-induced insulin resistance and reveals actionable targets for mucosal precision-based immunometabolic therapy.

Costa, Bruno: Charite.

- *The Microbiota Drives Epithelial–Stromal Crosstalk and Skin Barrier Homeostasis via ILC-Derived IL-22*

The epithelial barrier of the skin forms a major interface with the external environment of the body. Innate lymphoid cells (ILC) are sentinel leukocytes that support adaptation of tissues to environmental cues. However, the mechanisms by which dermal microbial signals maintain tissue homeostasis remain unclear. Here, we define how dermal commensals shape the organization and cytokine output of ILC, with IL-22 emerging as a central regulator of homeostasis in the skin.

Using genetic fate mapping, transcription factor reporters, spatial imaging, and single-cell RNA-sequencing, we showed that dermal ILC are stable, niche-specialized, tissue resident populations distributed across diverse microenvironments. These cells display mixed ILC3/ILC2 gene programs with RORγt lineage history, seed tissues early in life, and show sex-dependent abundance within lipid-rich stromal–epithelial units near the microbiota.

Manipulation of microbial complexity using germ-free, antibiotic-treated, monocolonized, and wildling mice revealed a graded relationship between microbial signals, and dermal ILC activity. Increased microbial complexity drives dermal ILC expansion and activation while promoting keratinocyte maturation, fibroblast differentiation and extracellular matrix deposition. Comparisons between commensal and virulent Staphylococcus species showed that microbial ecology dictates immune activation, with virulent strains disrupting structural programs supported by commensals.

IL-22 signaling activated a coordinated fibroblast program that enhanced trophic support for the epidermis, influenced key developmental and metabolic networks, and engaged circuits involved in lipid handling. This fibroblast response strengthened communication across stromal and epithelial compartments, supporting keratinocyte metabolism, differentiation, and overall barrier performance. In the absence of IL-22, these interconnected processes were disrupted, leading to impaired barrier integrity, altered tissue homeostasis, and reduced support for epidermal renewal. Together, these findings identify microbiota-driven ILC activity as a central organizer of dermal architecture and position IL-22 as a critical regulator of barrier resilience."

Golub, Rachel: Institut Pasteur/Université Paris Cité.

- *Neonatal ILC2s shape the vascular niche to enable postnatal establishment of HSC quiescence*

Innate lymphoid cells (ILCs) are central regulators of tissue homeostasis. Among them, ILC2s uniquely complete their differentiation within the bone marrow (BM), yet their contribution to the developing hematopoietic niche remains unclear. We investigated the role of BM-resident ILC2s during early postnatal life, the window preceding the establishment of a stable quiescent hematopoietic stem cell (HSC) pool around P21.

Genetic ablation of ILC2 results in a sustained reduction of the HSC compartment. This defect originates from impaired neonatal HSC survival and non cell-autonomous alterations of the microenvironment, indicating that ILC2s contribute to niche dependent mechanisms required for proper postnatal HSC maturation.

To define the molecular basis of these alterations, we performed single-cell RNA sequencing of stromal (MSC-like) and endothelial compartments at two weeks of age, a stage preceding full establishment of HSC quiescence. In parallel, we profiled ILC2s and hematopoietic progenitors to reconstruct niche interactions. Our analyses reveal an increased inflammatory signature in HSC and sinusoidal endothelial cells (sEC) in ILC2-deficient mice. We identify two distinct sEC clusters, only one of which exhibits a pro-inflammatory transcriptional program. Notably, the proportion of inflammatory sEC is markedly increased in the absence of ILC2.

Given that chronic inflammatory signaling compromises long-term HSC maintenance, these findings support a model in which neonatal ILC2s regulate vascular niche identity to enable the postnatal establishment of HSC quiescence. Ongoing work aims to define the molecular signals mediating crosstalk between ILC2s, endothelial cells, and HSC during this developmental transition. Collectively, our data position neonatal ILC2s as regulators of vascular niche maturation and early-life hematopoietic homeostasis, extending their functional scope beyond peripheral immunity.

Hatai, Shunya RIKEN

- *Microbiota-driven epithelial–ILC2 circuit programs barrier immunity to suppress colitis*

Appendectomy is epidemiologically associated with a reduced risk of ulcerative colitis (UC), yet the immunological mechanisms underlying this durable protection remain unclear.

Using a murine cecal resection (CR) model that recapitulates this protective effect, we investigated how surgical alteration of the cecal niche reshapes mucosal immunity. Strikingly,

CR established a stable, colon-specific type 2–biased immune state prior to inflammatory challenge. At steady state, CR mice exhibited selective expansion and activation of group 2 innate lymphoid cells (ILC2s) in the colon, accompanied by elevated IL-13 production, without global immune suppression.

This preconditioned immune landscape conferred marked resistance to DSS-induced colitis, reducing inflammatory cytokine production and myeloid infiltration while reinforcing mucus barrier integrity. Genetic ablation and neutralization studies demonstrated that ILC2s and IL-13 were indispensable for CR-mediated protection, whereas T cells and IL-5 were dispensable, identifying ILC2-derived IL-13 as the dominant effector of barrier reinforcement.

Mechanistically, CR induced persistent tuft cell hyperplasia and elevated epithelial IL-25 expression specifically in the colon, but not in the small intestine. Tuft cell deficiency or IL-25 deletion abolished ILC2 activation and eliminated disease protection, establishing a colon-restricted tuft cell–IL-25–ILC2 axis as the core protective circuit.

Microbiota transfer and co-housing experiments revealed that CR-induced protection was transmissible and dependent on microbial reshaping. Metabolomic analyses further indicated remodeling of the intestinal metabolic environment, and administration of selected microbiota-derived metabolites partially recapitulated tuft cell expansion and barrier enhancement.

Collectively, our findings reveal that appendectomy-like structural perturbation durably reprograms colonic ILC2 responsiveness through microbiota-dependent metabolic cues, establishing a pre-inflammatory type 2–biased barrier state resistant to inflammatory challenge.

Ismailova, Nailya: McGill University

- *Group 2 innate lymphoid cells are critical to drive lung-resident allergic IgE responses through an IL-33-mediated c-Rel-dependent 4-1BBL signalling axis*

Primary exposure to allergens establishes allergen-specific immunoglobulin E (IgE) antibodies which mediate effector cell degranulation, leading to airway pathology. Recurrent allergen exposure drives repeated allergic symptoms, a response typically associated with immunological T and B cell memory. However, IgE-expressing B cells fail to form long-lasting memory B cells. Rather, CD4+ T helper 2 (Th2) cells provide IL-4 to promote class switching of lung-resident immunoglobulin G (IgG) memory B cells to maintain long-term allergen-specific IgE responses during secondary allergic exposure. However, the molecular drivers and cellular interactions promoting tissue-resident Th2 cells and IgE responses locally during secondary allergic exposure remain ill-defined.Tissue resident group 2 innate lymphoid cells (ILC2s) have been recently shown to exert not only key roles in orchestrating innate type 2 immune responses but also interact with T and B cells.We therefore hypothesized that ILC2s regulate local tissue-resident type 2 adaptive immune responses during allergen challenge. Bulk RNA and ChIP sequencing of IL-33-activated ILC2s revealed that c-Rel, an NF-κB subunit, promotes distinct transcriptional changes, including upregulation ofTnfsf9, encoding 4-1BBL, a T cell co-

stimulatory molecule that promotes T cell survival, expansion, and acquisition of effector phenotypes. Using c-Rel-deficient mice, we demonstrated that 4-1BBL expression induced by IL-33 on ILC2s is largely c-Rel-dependent. High-parameter spectral flow cytometry revealed that during allergic airway inflammation, ILC2s are the sole pulmonary cell type expressing 4-1BBL and T cells express high 4-1BB levels. Using an ILC2-specific 4-1BBL-deficient mouse model with Alternaria alternata allergen, we observed that 4-1BBL expression by ILC2s did not impact innate allergic response induction. In contrast, lung immunophenotyping revealed that ILC2 4-1BBL expression is required to establish tissue-resident and systemic allergen-specific IgE recall responses. Together, these data demonstrate that IL-33 activation of ILC2 induces a c-Rel-dependent signaling cascade that sustains tissue-resident pulmonary CD4+ T cells and allergen-specific IgE responses..

Khijakadze, Davit: University Of British Columbia.

- *Chronic Allergic Lung Inflammation Drives Emphysema Development via ILC2 plasticity and IL-17A production*

Group 2 innate lymphoid cells (ILC2s) are activated by epithelium-derived alarmins such as IL-33, IL-25, and thymic stromal lymphopoietin (TSLP). Once activated, ILC2s proliferate and produce the type 2 cytokines IL-5 and IL-13.These cytokines induce eosinophilia and mucus hyperproduction, respectively, leading to allergic inflammation. However, the role of ILC2s in chronic allergic inflammation remains unclear.

We developed a mouse model of chronic allergic lung inflammation induced by repeated intranasal injections of the protease allergen papain. This model allowed us to investigate the effects of chronic allergen exposure on lung ILC2s in wild-type, RAG-KO, Rorct-KO, and IL-33-KO mice. Flow cytometry, ELISA, and lung histology analysis were used to identify involved populations and cytokines.

During the first 3 weeks of treatment, ILC2s significantly expanded and produced type 2 cytokines, inducing lung eosinophilia and mucus production. However, after 5 weeks of chronic allergen exposure, the number of ILC2s decreased; they were less responsive, proliferated minimally, and produced fewer cytokines. Unexpectedly, group 3 innate lymphoid cells (ILC3s) producing IL-17A expanded. Additionally, a subset of ILC2s produced both IL-5 and IL-17 and became GATA3+Rorγt+, implying that ILC2s are capable of producing IL-17 and may also transdifferentiate into ILC3s. Additionally, H&E staining of lung tissue from wild-type mice after 5 weeks of treatment revealed peribronchial inflammation and emphysema, suggesting a role for ILC2s and ILC3s in emphysema development. Analysis of RAG-KO mice further supported this, as emphysema was also observed in these mice.

These findings suggest that chronic allergic lung inflammation induces IL-17 production by ILC2s in addition to their transdifferentiation into IL-17A-producing ILC3s, ultimately driving

emphysema development. These processes might be responsible for COPD development in some patients with chronic asthma.

Khijakadze, Davit: University Of British Columbia

- *Plasticity of ILC2s Toward an IL-17–Producing Phenotype in Chronic Allergic Inflammation and Emphysema*

Group 2 innate lymphoid cells (ILC2s) are activated by epithelium-derived alarmins such as IL-33, IL-25, and thymic stromal lymphopoietin (TSLP). Once activated, ILC2s proliferate and produce the type 2 cytokines IL-5 and IL-13. These cytokines induce eosinophilia and mucus hyperproduction, respectively, leading to allergic inflammation. However, the role of ILC2s in chronic allergic inflammation remains unclear.

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These findings suggest that chronic allergic lung inflammation induces IL-17 production by ILC2s and their transdifferentiation into IL-17A-producing ILC3s, ultimately driving emphysema development. These processes might be responsible for COPD development in some patients with chronic asthma.

Kuo, Grace (I-Chih): University of British Columbia

- *Pluripotent Stem Cell-Derived Type 2 Innate Lymphoid Cells Exhibit Potent Antitumor Activity Against Pancreatic Cancer Cells*

Introduction: Innate lymphoid cells (ILCs) are tissue-resident immune cells that share similar developmental and transcriptional programs with T cells but lack antigen-specific receptors. Functionally, they act as frontline, immediate effectors to prime appropriate responses prior to the expansion of antigen-specific T cells and combating infections. Like T cells, they also can exhibit anti-tumor functions through cytotoxicity, cytokine secretion, and immune cell recruitment. Thus, leveraging these functions could offer a novel avenue for cancer immunotherapy and, potentially, overcome some of the limitations of conventional T cell-based approaches in treating solid tumors.

Methodology: We developed a differentiation workflow to generate ILC2-like cells from human pluripotent stem cells (hPSCs). Cytokine supplementation, ligand coating, and small molecule supplementation were optimized to promote the development of functional, cytokine-secreting ILC2s under feeder-free and animal-component-free conditions.

Results: Flow cytometry analyses revealed that approximately 50% of CD45+Lineage- cells emerging from our protocol are mature ILC2-like cells and secrete canonical type 2 cytokines, including IL-5 and IL-13. These cells display metabolic profiles and immunophenotypic characteristics comparable to peripheral blood-derived ILC2s. Upon IL-33 stimulation, the isolated iPSC-derived ILC2s induce apoptosis in pancreatic cancer cells through the DNAM1-CD112/CD155 axis and significantly inhibit tumor cell proliferation. These effects were confirmed using vital co-culture imaging and MTT assays. Transwell experiments and Olink inflammatory-mediator profiling identified both direct cytotoxic interactions and soluble factors as contributors to cancer cell killing. Preliminary xenografting studies in NSG mice are ongoing to confirm the antitumor activity and persistence of PSC-derived ILC2s in vivo.

Conclusion: This work establishes a scalable platform for generating functional ILC2s from pluripotent stem cells, enabling detailed studies of ILC2 development and function in humans. These findings lay the groundwork for advancing ILC-based immunotherapies. Additionally, PSC-derived ILC2s offer a versatile tool with therapeutic applications in chronic inflammatory and autoimmune diseases.

Lopez Espinoza, Alejandra: University of Washington

- *Iron-deficiency impairs host resistance to intestinal helminth infection*

Soil-transmitted helminth infections affect more than 1.5 billion people, often in regions where anemia, due to dietary insufficiency and malaria, is common. How host iron levels influence the type 2 immune and epithelial response that promotes helminth expulsion is unclear. To assess how dietary iron impacts critical group 2 innate lymphoid cell (ILC2) and CD4+ T cell responses that drive anti-helminth responses, mice were fed an iron-sufficient (IS) or -deficient (ID) diet prior to infection with the helminth Nippostrongylus brasiliensis (Nb). At seven days post Nb-infection, we observed defects in worm clearance in ID mice compared to IS mice, and mature

worms from ID hosts had higher ATP levels than those from IS hosts, suggesting that host ID does not impair worm fitness. Five days post Nb-infection, when ID and IS hosts had similar worm burdens, ID mice had reduced recruitment of ILC2s into the mesenteric lymph node (mLN). Additionally, ID-Nb infected mice had decreased accumulation of IL-4+CD4+ T cells in the small intestine compared to IS hosts. To examine the role of intracellular vs. extracellular iron in this context, we treated mice with extracellular iron chelator deferoxamine (DFO) or the membrane-permeable intracellular iron chelator deferiprone (DFP). Only DFP-treated mice showed increased worm burden seven days post Nb-infection and reduced recruitment of GATA3+ILC2 and GATA3+Th2 in the mLN compared to DFO-treated and control infected mice. These findings demonstrate that depletion of intracellular iron delays ILC2 recruitment and the downstream Th2 response, ultimately resulting in defects in worm expulsion. These findings provide novel insights on how dietary iron supports type 2 cytokine-mediated immune responses against helminth infection and highlight its potential as an intervention for type 2 immunity.

Luo, Xinxin: Amsterdam University Medical Center

- *Nuclear Receptor LXR as a Checkpoint in Tuft Cell-ILC2 Circuit Modulates Intestinal Type 2 Immunity*

The bidirectional communication between group 2 innate lymphoid cells (ILC2) and tuft cells is crucial in type 2 mediated inflammation, including parasitic infections and allergies. While intestinal tuft cells are known to initiate type 2 immunity, the endogenous calibration of their effector function remains poorly understood. This study identifies liver X receptor (LXR), a nuclear receptor that senses endogenous oxysterols, as an epithelial-intrinsic checkpoint controlling tuft cell numbers and setting the threshold for type 2 immune responses. In vivo, ectopic LXR activation decreased tuft cell and ILC2 numbers in the small intestine, while LXRβ deficiency, but not LXRα, led to their expansion. LXR activation acted upstream of IL-25 production by tuft cells, as demonstrated by the rescue of ILC2 and tuft cell numbers through recombinant IL-25 administration. Genetic ablation of LXR in intestinal epithelium confirmed its epithelial-intrinsic necessity. Consequently, LXR activation attenuated the tuft-ILC2 amplification circuit, impairing anti-helminth defense but protecting against experimental food allergy in mice. These findings identify LXR as a central modulator of mucosal type 2 immunity in the intestine, offering potential therapeutic targets for type 2 inflammatory disorders.

Matthew Hepworth: University of Manchester

- *A heparan sulfate glycocalyx supports maintenance of tissue-resident group 2 innate lymphoid cells.*

Mammalian health is underpinned by long-lived tissue-resident immune cells, including Innate lymphoid cells (ILC). ILC are enriched within barrier sites and sense contextual cues from the

host and environment to restore tissue homeostasis. Maintenance of ILCs requires competition for essential survival cues which occurs within discrete tissue microenvironments via interactions with non-hematopoietic cells. However, the mechanisms utilized by ILCs to compete for these cues and persist within tissue niches remain poorly understood. Here we demonstrate group 2 ILCs (ILC2) are enriched for a signature associated with the synthesis of the glycosaminoglycan heparan sulfate (HS). Using cross-disciplinary approaches we reveal ILC2 are coated with a cell-surface HS glycocalyx that plays critical roles in supporting cell survival. Cell-intrinsic deletion of two distinct enzymes critical for HS synthesis resulted in markedly reduced ILC2 numbers and impaired effector responses against helminth infection. Mechanistically, we reveal HS acts to facilitate optimal binding and signalling of the survival cytokine IL-7 to ensure ILC2 population maintenance with the adventitial cuff of the airways. Together these findings reveal an unappreciated role for immune cell-associated glycosaminoglycan in barrier immunity and ILC regulation in barrier tissues.

Mattiola, Irene: Charité- Universitätsmedizin Berlin

- *Group 3 innate lymphoid cells control endothelial cell remodelling in cancer*

Group 3 innate lymphoid cells (ILC3) are tissue-resident interleukin (IL)-22-producing innate lymphocytes regulating tissue homeostasis. ILC3 are found in tumors at barrier tissues, but how they interact with components of the tumor microenvironment remains largely elusive. Here, we set out to identify ILC3-dependent tissue modules relevant for skin cancer. We observed that high ILC3 frequencies in skin cutaneous melanoma patients correlated with reduced overall survival. In preclinical mouse models of melanoma, lack of ILC3 or IL-22 substantially delayed tumor growth, suggesting a pro-tumoral function of ILC3 mediated by IL-22. IL-22-producing ILC3 located in the tumor fibrovascular niche and tumor endothelial cells expressed high levels of IL-22 receptor. Genetic deletion of the IL-22 receptor specifically on endothelial cells impeded tumor growth. We obtained a single cell RNA-seq atlas of tumor endothelial cells fromIl22+/+andIl22-/-mice revealing that IL-22 is required for the maintenance of a previously undescribed population of tumor endothelial cells endowed with stem-like and de-differentiated features underpinned by enhanced YAP/TAZ activation. IL-22 signaling in differentiating tumor endothelial cells led to aberrant and dysfunctional vessels and low leukocyte infiltration of the tumor, particularly of effector innate lymphocytes endowed with anti-tumor effector functions. Our findings demonstrate that IL-22-producing ILC3 exert pro-tumor functions by regulating tumor angiogenesis and limiting anti-tumor immunity. We identified a previously undescribed ILC3-endothelial cell module in cancer that can be targeted by novel therapeutic approaches.

Misawa, Takuma: RIKEN

- *ILC3s rewire lipid transport via the portal vein and regulate systemic metabolism*

Among dietary fatty acids (FAs) absorbed in the small intestine (SI), short- and medium-chain FAs are directly transported to the liver via the portal vein (PV). In contrast, long-chain FAs (LCFAs) are incorporated into chylomicrons and transported to peripheral tissues through intestinal lymphatics, with minimal entry into the PV. Despite reports detecting dietary LCFAs in the PV, the mechanisms and physiological significance of this pathway have remained unclear. Here, we identify group 3 innate lymphoid cells (ILC3s) as key regulators of PV-dependent LCFA transport and systemic metabolic homeostasis. Feeding induces vasoactive intestinal peptide (VIP) release in the SI, which stimulates ILC3s to produce vascular endothelial growth factor A (VEGF-A). VEGF-A, in turn, induces the formation of gaps within intestinal capillaries, through either by endothelial junctional openings or fenestrations, permitting chylomicrons to enter the portal circulation. Genetic ablation of ILC3s, VIP receptor 2, or ILC3-specific VEGF-A expression markedly impairs PV-mediated, but not lymphatic-mediated, transport of dietary LCFAs in mice. Impaired PV-dependent lipid transport reduces adipose tissue mass under both normal and high-fat diet conditions and compromises ketone body production during fasting, leading to increased mortality under repeated nutrient deprivation. Together, these findings uncover an unappreciated portal route for dietary lipid transport and reveal a central role for ILC3s in coupling feeding cues to vascular remodeling, lipid trafficking, and metabolic adaptation essential for survival during fasting.

Neves, Joana F: King's College London

- *Human type 3 innate lymphoid cells shape intestinal fibroblast phenotype and extracellular matrix remodelling via TNF-α*

Type 3 innate lymphoid cells (ILC3) are abundant in the human gut and play a key role in maintaining tissue integrity. While their interactions with epithelial cells are well described, their influence on fibroblasts and extracellular matrix (ECM) remodelling remains poorly defined. Dysregulation of ILC3 is a feature of chronic inflammatory disorders such as inflammatory bowel disease (IBD), where tissue remodelling is often abnormal. Here, we investigated the mechanisms governing human intestinal ILC3-stromal interactions.

We generated human iPSC-derived intestinal organoids and co-cultured them with blood-derived ILC precursors, which differentiated into mature, cytokine-producing ILC3 with intestinal-like transcriptional features. These ILC3 were subsequently co-cultured with iPSC-derived intestinal organoids containing both epithelial cells and fibroblasts.

ILC3 exposure induced global transcriptional changes in fibroblasts, including upregulation of immune signalling and tissue remodelling pathways. Fibroblasts from co-cultures displayed reduced α-smooth muscle actin and fibroblast activation protein, and decreased collagen I/III deposition, consistent with transcript-level changes. Concurrently, ILC3 increased matrix metalloproteinase-1 expression and secretion in both stromal and epithelial compartments.

Upstream regulator analysis on identified TNF-α as a central mediator of these effects. Exogenous TNF-α recapitulated the ILC3-induced fibroblast phenotype, while TNF-α neutralisation reversed it. Analysis of public datasets revealed reduced TNF-α expression in intestinal ILC3 from patients with active IBD, suggesting a context-dependent role in ECM regulation.

Together, these findings identify a previously unrecognised role for human ILC3 in shaping fibroblast phenotype and ECM remodelling through TNF-α–dependent mechanisms, highlighting a novel immune–stromal axis relevant to intestinal tissue homeostasis and chronic inflammatory disease.

Poholek, Amanda: University of Pittsburgh

- *IL-9 and Blimp-1 protects the transcriptional identity of group 2 innate lymphocytes in allergic asthma*

Allergic asthma is driven by type 2 immune responses, including type 2 innate lymphoid cells (ILC2s). Although ILC2s are activated by the tissue alarmins IL-33 and IL-25, these signals do not intrinsically enforce type 2 identity, and the mechanisms that maintain type 2 cytokine expression remain unclear. Here we show that allergen-induced IL-33 and IL-25 rapidly induce IL-9, which in turn upregulates the transcriptional repressor Blimp-1 in ILC2s. Blimp-1 sustains type 2 immunity by directly repressing type 1 inflammatory programs, including expression of IFNg and TNF. Deletion of Blimp-1 in ILC2s increased type 1 cytokine production and reduced IL-5 and IL-13 expression, eosinophil recruitment, and mucus production in the lung. In contrast, IL-9 expression was enhanced in the absence of Blimp-1, leading to increased mast cell recruitment. Together, these findings identify Blimp-1 as a key regulator of ILC2 transcriptional fidelity that stabilizes type 2 inflammation while constraining divergent inflammatory programs during allergic responses.

Raghunathan, Kiruthiga: Olivia Newton John Cancer Research Institute

- *Tumor – Educated ILC2s Prime Distant Organs for Metastasis in Gastric Adenocarcinoma*

Gastric Cancer remains one of the most lethal malignancies worldwide, with poor prognosis largely driven by late – stage diagnosis and limited therapeutic options for metastatic disease. Innate immune cells have a well-established role in inflammation and mucosal immunity, but their role in organ specific metastasis is less understood. The function of innate lymphoid cells type - 2 (ILC2), in shaping the pre-metastatic niche remains underexplored.

This study investigates tumor-educated ILC2s as early orchestrators of liver metastasis in gastric cancer. Using an orthotopic mouse model of gastric adenocarcinoma with liver metastases, we employed genetic mouse models deficient in activated ILC2 (ILC2IL5KO) mice to dissect tumor

educated ILC2 functions at days 10, 20, 30, and 50 post-transplantations of tumor, revealing their roles in pre-metastatic niche maturation, stromal remodeling, immunosuppression, and metastasis progression.

ILC2 frequency was markedly increased in gastric tumors, peripheral blood and early metastatic niches and robust development of macroscopic liver metastases. Conversely, ILC2IL5KO mice showed reduced primary tumor burden, fewer circulating ILC2s, reduced metastatic niches and a complete absence of macroscopic liver metastases.

Multiplex immunohistochemistry identified ILC2-enriched clusters in the liver that activate Kupffer cells to a M2-like phenotype and recruit CD103+ Tregs, creating immunosuppressive pre-metastatic niches that preferentially attract epithelial-to- mesenchymal transition (EMT) like cancer cells for colonization. Cytokine profiling revealed elevated IL13 production by ILC2s contributing to a pro-metastatic microenvironment in the liver. Blocking ILC2 trafficking and effector cytokine function significantly curtailed metastatic progression. We used bulk RNA sequencing of tumor educated ILC2s to investigate the underlying mechanisms for ILC2 trafficking and metastasis progression.

In conclusion, tumor educated ILC2s are critical early drivers of premetastatic niche formation and metastatic seeding in gastric cancer. These findings uncover a previously unrecognized innate immune axis linking primary solid tumors to distant organ metastasis and highlight ILC2 directed interventions as a promising strategy to limit metastatic spread.

Ren, Deshan: Nanjing university

- *Distinct ontogeny of functional divergent human uterine natural killer cells in vivo*

Uterine natural killer (uNK) cells constitute a heterogeneous population essential for healthy pregnancy, yet their developmental origins in humans remain unclear. To address this, we employed human immune system mice established on an NCG-X background with human interleukin-15 knock-in, a model that supports robust uNK cell development and allows pregnancy. Using this system, we demonstrated that uNKs arose through two ontogeny pathways: CD56brightCD16– peripheral blood NK (CD56bri PBNK) cells and innate lymphoid cell progenitors (ILCPs). These two lineages give rise to phenotypically and functionally distinct uNK subsets, which can be distinguished by CD2 expression. CD56bri PBNK derived CD2+ uNK cells exhibited cytotoxic features and favor immune surveillance, whereas ILCP derived CD2– uNK contributed to fetal growth through secretion of pro-angiogenic factors and cytokines that drive trophoblast invasion. This study establishes a dual-origin framework for uNK heterogeneity, highlighting the critical role of ILCP derived CD2– uNK in fetal development.

Ricardo-Gonzalez, Roberto: University of California San Francisco

- *Demodex mites drive skin-like CD103+ ILC2s into the lung*

Proper lung function involves tissue-resident immune cells that participate in homeostasis but also protect against further multiple perturbations. Although the "gut-lung" axis has been described, our study addressed a novel paradigm by which skin ILC2s (skILC2) become educated post-birth during interactions with a commensal hair follicle mite, Demodex, proliferate, exit their tissue of developmental origin, and transit to the lung, termed ex_skILC2s. Dissecting how ex_skILC2s impact the lung microenvironment may reveal novel mechanistic ways to safely promote tissue tolerance to allergens and infectious challenges in preemptive ways that open novel therapeutic strategies for improving lung health.We used a combination of spectral flow cytometry, RNA-seq, tissue imaging, parabiosis, infectious (Nippostrongylus (Nb), Influenza), and allergic (OVA, alternaria) models to test our hypothesis. We show that WT mice infected with Demodex triggered a distinct skin ILC2 subset with a mixed type 2-3 cytokine repertoire, which expands, egresses the skin, enters the blood, and infiltrates into lungs, where they adapt and establish long-term residency. We demonstrate ex_skILC2s in lung are the first group of ILC to be activated upon challenge by the migratory helminth, Nippostrongylus brasiliensis (Nb). In the chronic phase, ex_skILC2s synergize with lung resident ILC2s (nILC2s), newly recruited gut inflammatory ILC2s (iILC2s), and de novo differentiated ILC precursors (ILCPs), to reshape the lung ILC2 landscape. We found that the presence of ex-skILC2s and their distinct activation kinetics play a role in promoting lung tissue repair during the resolution of inflammation after Nb infection. Our study highlights that the accumulation of ex_skILC2s complements the natural lung immune cell repertoire in ways that enhance the homeostasis and resilience of the organ to diverse post-birth perturbations.

Riedel, Maximilian: Charite - Universitätsmedizin Berlin

- *Stromal cell–derived IL-7 as a regulator of tissue-specific innate lymphoid cell development and maintenance*

Interleukin-7 (IL-7) is a key cytokine regulating innate lymphoid cell (ILC) development, survival and proliferation as shown by studies using IL-7-/- or IL7Ra-/- in which ILC subsets were markedly reduced. Yet the sites that constitute optimal microenvironments for ILC differentiation and maintenance, and the specific contribution of stromal cell–derived IL-7 to tissue ILC niches remains incompletely understood. To address this, we conditionally deleted IL-7 in PDGFRα$^+$ stromal cells and examined the consequences for ILC development and/or maintenance.

In the bone marrow as the central organ of postnatal hematopoiesis, loss of IL-7 from PDGFRα$^+$ stromal cells resulted in a moderate but incomplete reduction in ILC2 progenitors, indicating that additional stromal sources contribute to maintaining the progenitor niche and suggesting that IL-7 production is shared among multiple mesenchymal cell populations.

We next investigated whether peripheral ILC2 pools depend on tissue-specific IL-7 environments and analyzed organs enriched in resident ILC2 populations, including lung, adipose tissue, small intestine, and mesenteric lymph nodes. While intestinal ILC2 frequencies were unchanged, IL-7 deletion in PDGFRα^{+} stromal cells caused tissue-dependent alterations in ILC2 abundance in the remaining organs, with adipose tissue showing the strongest effect. These findings suggest that the establishment and persistence of ILC2 in peripheral tissues are governed by the local microenvironment and highlight that stromal IL-7 niches serve a unique role in an organ-specific manner. Longitudinal analyses further revealed that these tissue-specific effects differ across embryonic, early postnatal and adult stages, indicating organ-dependent kinetics in the establishment and maintenance of peripheral ILC2 populations.

Collectively, our findings indicate that ILC2 homeostasis across organs relies on tissue-specific IL-7 sources, with certain tissues showing a stronger dependence on stromal cell–derived IL-7 to maintain local ILC2 populations over time.

Sheh, Lei: Shanghai Jiao Tong University

- *Metabolic and epigenetic control of neuro-immune crosstalk by mTORC1 in ILC2s*

Group 2 innate lymphoid cells (ILC2s) integrate tissue-derived cytokine and neuronal signals to drive type 2 inflammation; however, the intracellular mechanisms that coordinate these inputs remain incompletely defined. Here, we demonstrate that mechanistic target of rapamycin complex 1 (mTORC1) functions as a central metabolic checkpoint governing neuroimmune responsiveness in lung ILC2s during allergic inflammation.

We observe enhanced mTORC1 activation in lung ILC2s under allergic inflammatory conditions. Conditional deletion of Raptor, an essential component of mTORC1, markedly attenuates IL-5 and IL-13 production and protects mice from allergic airway inflammation. Pharmacologic inhibition with rapamycin similarly suppresses ILC2 effector function and disease severity.

Mechanistically, mTORC1 promotes epigenetic remodeling at the neuromedin U receptor 1 (NMUR1) locus, resulting in increased chromatin accessibility and augmented NMUR1 expression. This metabolic-epigenetic coupling enhances ILC2 responsiveness to neuromedin U (NMU), thereby amplifying neuronal–immune communication. While NMUR1 upregulation contributes substantially to mTORC1-driven activation, additional downstream pathways also participate in regulating ILC2 effector programs.

Collectively, our findings identify mTORC1 as an integrative hub linking environmental cues sensing, epigenetic programming, and neuronal signal responsiveness in ILC2s. These results provide a mechanistic framework for how metabolic pathways shape neuroimmune crosstalk to optimize ILC2 function in tissue inflammation.

Trabanelli, Sara: University of Geneva

- *Tumor-derived steroids convert NK cells into pro-fibrotic immunosuppressive effectors in human cancer*

Although high-grade serous ovarian carcinoma (HGSOC), the most frequent and aggressive histotype of ovarian cancer, typically exhibits poor responses to immune checkpoint inhibitors, this limited efficacy has been attributed in part to dominant innate immune-mediated suppression. Natural killer (NK) cells are frequently detected in HGSOC and express high levels of the glucocorticoid (GC) receptor, yet how tumor-associated steroids shape NK cell function and tissue organization remains unclear. Here, we found that GCs are enriched in malignant ascites of HGSOC patients and reprogram GC receptor-expressing NKs from cytotoxic to tolerogenic states. Endogenous GCs limit NK effector functions while inducing secretion of amphiregulin (AREG), a key mediator of tissue remodeling and fibrosis. Spatial multiomic imaging revealed that the AREG receptor EGFR is mainly expressed by fibroblasts, and that NK cells and fibroblasts colocalize in fibrotic regions at the tumor periphery. Distance mapping revealed that T cells are excluded from these NK-fibroblast niches and remain positioned away from the tumor core, consistent with the immune-excluded phenotype frequently observed in HGSOC.These findings suggest that GC-induced AREG+ NKs cooperate with fibroblasts to establish a physical and functional barrier to T cell infiltration. Importantly, Relacorilant, a first-in-class selective GC receptor antagonist that improves survival in platinum-resistant ovarian cancer, restored NK cytotoxic function and reduced fibroblast activation. This phenomenon extended across multiple cancer types and appeared tumor-associated, as ascites from cirrhotic patients lacked this activity. Together, our findings uncover a previously unrecognized steroid-driven mechanism of immune evasion in human cancer with direct therapeutic implications.

Zhu, Jinfang: NIAID, NIH

- *Transcriptional regulation of cytokine expression in type 2 lymphocytes*

Type 2 innate lymphoid cells (ILC2s) and Th2 cells play an important role in regulating type 2 immune responses by producing type 2 effector cytokines including IL-4 and IL-13. While GATA3, the master transcription factor for ILC2 and Th2 cell differentiation, is essential for type 2 cytokine production, GATA3 expression itself even at high levels is not sufficient to induce transcription of either Il4 or Il13 under certain circumstances. To better understand the transcription factors that regulate IL-4 and IL-13 expression during the differentiation of these type 2 lymphocytes, we performed CRISPR-Cas9 screens in vitro with a retroviral library targeting 2,394 TFs and cofactors. We discovered a previously unappreciated role for Cbfβ (core binding factor beta) in promoting IL-13 expression. In the same screen, NFAT1 was found to preferentially induce IL-4 expression. The effect of Cbfβ on type 2 cytokine production which were confirmed both in vitro and in vivo was independent of GATA3 induction. We also found that early but not late deletion of Cbfβ in Th2 cells affected IL-13 production. To further

explore the mechanism by which Cbfβ regulates the expression of IL-13, we used DNase I-Seq to assess chromatin accessibility. The results showed that knocking out Cbfβ reduced the accessibility at the HS-2 site in the Il13 promoter. Cbfβ ChIP-Seq in Th2 cells showed a binding peak in the HS-2 site. DNA looping between HS-2 and other enhancers were reduced when knocking out Cbfβ in Th2 cells from our Hi-TrAC analysis. These results indicating that Cbfβ regulate IL-13 expression directly by binding to Il13 promoter and bringing together enhancers to the Il13 promoter to facilitate Il13 transcription. We are currently studying whether Cbfβ also regulates IL-13 expression in ILC2s. In the meantime, we discovered that the transcription factor Bhlhe40 induces GM-CSF but represses IL-10 in ILC2s, which modulates inflammatory and anti-inflammatory properties of ILC2s.

Zutautas, Katherine B.: Queen's University

- *Group 2 innate lymphoid cells regulate interleukin-33 driven inflammation in endometriosis*

Endometriosis (EM) is an estrogen-dependent chronic inflammatory gynaecological disease impacting 10% of women globally. The alarmin, interleukin-33 (IL-33), is significantly elevated in the plasma, peritoneal fluid (PF), and lesions of EM patients compared to healthy controls and positively correlated with disease severity. IL-33 treatment in a mouse model of EM exacerbates cardinal features of disease (inflammation, fibrosis, vascularization) and group 2 innate lymphoid cells (ILC2s) have emerged as a key cell type in orchestrating these effects. We aim to mechanistically establish that ILC2s are required for IL-33 driven EM pathophysiology to solidify the IL-33/ILC2 axis as a potential therapeutic target. Interrogation of components in the IL-33/ILC2 axis at the mRNA and protein level was performed across EM patient and control samples (plasma, PF, tissue -lesion, endometrium). EM lesions show enrichment in the IL-33/ST2 axis as gene expression of IL-33 and its receptor ST2 are significantly elevated compared to healthy control endometrium and SIGIRR, an inhibitor of IL-33/ST2 signaling, is significantly reduced in EM lesions. Through utilization of EM induced ILC2 competent (C57Bl6) and specific ILC2 deficient mice (Nmur1iCre-eGFP Id2fl/fl), we demonstrate that ILC2 deficient mice treated with IL-33 lack characteristic type 2 inflammatory cytokines (eotaxin, IL-5) and accompanying eosinophil and macrophage recruitment in the PF. Further, bulkRNA sequencing demonstrated a distinct transcriptional signature of EM lesions deficient in ILC2s, highlighting the critical role of ILC2s in shaping the lesion microenvironment. Finally, adoptive transfer of ILC2s through intraperitoneal injection was unable to rescue the blunted type 2 response and altered immune recruitment, suggesting a potential role for tissue specific ILC2s in mediating the IL-33 driven inflammatory response. Our studies reveal that ILC2s are a critical cell type in EM IL-33 driven inflammation and associated pathophysiology. Studies in mice are in progress investigating ST2 blockade as a potential therapeutic strategy for EM.

ILC6 2026 Abstracts:

Poster Presentations- Alphabetical by Submitter

Acharya, Belize: German Cancer Research Cancer (DKFZ)

- *Regulation of Brain Tumor Immunity by Innate Lymphoid Cells*

Innate lymphoid cells (ILCs) constitute a small, predominantly tissue-resident population of the innate immune system. They exert a broad range of immunoregulatory and immunostimulatory functions, including defense against intra- and extracellular pathogens as well as antigen presentation. Their therapeutic potential as immunomodulatory targets is increasingly recognized across diverse disease forms, including infection, inflammation, and cancer. However, the role of ILC1–3 in glioblastoma (GB) has remained largely unexplored, particularly in light of their absence from the steady-state brain parenchyma.

In this study, we firstly demonstrate the presence and abundance of ILC1–3 in both murine and human GB using flow cytometry. We further show that antibody-mediated depletion of NK cells and ILC1 via anti–asialo-GM1 treatment results in prolonged survival in murine GL261-SIINFEKL brain tumor models, providing initial evidence for a potential role of these cells in regulating tumor progression.

Moreover, we present longitudinal single-cell RNA sequencing data from murine GL261-SIINFEKL brain tumors including immune cells derived from the tumor site as well as from the corresponding gut, an organ characterized by a relatively high abundance of ILCs under steady-state conditions. This approach enables a detailed analysis of ILC subtypes across different tissues and time points under the condition of a primary brain tumor. We thereby provide first insights into glioblastoma-specific phenotypic features and associated functional properties of ILCs. Collectively, these findings uncover a previously unrecognized component of the GB immune microenvironment and contribute to a deeper understanding of regulatory mechanisms operating within this highly immunosuppressive tumor entity.

Bassett, John Karolinska Institutet

- *Spatial disorganization of innate and adaptive lymphocytes in colonic tissue layers in Crohn's disease*

The intestinal immune system maintains a balance between host defense and tolerance. In Crohn's disease (CD)this balance is disrupted resulting in patchy lesions interspersed with non-affected areas. To improve the development of CD treatments, we examined lymphocyte dysregulation across the layers of the non-affected colonic wall of CD and non-CD controls.

We used microdissection to segregate the anatomical layers (epithelia, mucosa, and submucosa) of macroscopically non-affected colon resection material from four patients with CD and from four non-CD control patients. We followed this with multiomic single-cell sequencingto spatially resolve T cell, NK cell, and innate lymphoid cell populations across these anatomical compartments.

Our analysis recapitulates established patterns of lymphocyte spatial organization both in CD and non-CD controls and identifies the submucosa as a distinct and previously underexplored immunological niche.

Across the different layers, the greatest number of Th1 cells are found in the mucosa, but we find a reduction of Th1 cells in the CD mucosa compared with the controls. In the CD submucosa we identify an accumulation of memory CD4+ T cells.

Within the CD8+ compartment, the mucosal layer harbors higher proportions of effector memory T cells whereas CD8+ tissue-resident memory (TRM) cells are most abundant within the epithelial layer. In CD, intraepithelial CD8+ TRM are reduced in frequency and display increased GNLY expression. Concurrent in the CD state, highly activated CX3CR1^{+} CD8+ T cells accumulate in the intraepithelial layer and submucosa. Lastly, we identify an influx of naïve CD8+ T cells into the CD submucosa.

ILC3 populations are predominantly localized to the mucosa in both settings, consistent with established distributions. However, we observe that submucosal ILC3 constitute a substantive population and, in CD, undergo a distinct transcriptional shift.

Our findings show the layer-specific reorganization of innate and adaptive lymphocyte populations extending beyond overtly inflamed lesions, highlighting the submucosa as a previously underappreciated site of immunological remodeling.

Boyles, Christabel: University of Cambridge

- *Natural killer cells mediate the efficacy of conventional therapies in primary and metastatic cancer*

Despite advances in personalised medicine and immunotherapy the standard of care for many cancer patients remains chemo- or radiotherapy. Crucially, the efficacy of these therapies hinges on immune function. While priming of adaptive CD8 T cells can mediate anti-tumour immune responses after conventional therapy, the relative importance of innate natural killer (NK) cells remains unclear. Using orthotopic mouse models we demonstrate that NK cell function contributes significantly to either chemo- or radiotherapy mediated control of ovarian cancer or lung metastases, respectively. Moreover, we assessed the effects of chemotherapy on endogenous tissue-resident immunity at sites of metastatic seeding, which revealed localised NK cell engagement. Using multi-omic single-cell and spatial approaches we infer

how carboplatin modulates the metastatic niche. Using IL-15/IL-15Rα therapy, we dynamically enhanced the local density of tumour-infiltrating cytotoxic lymphocytes, including NK cells and CD8 T cells, which significantly improved chemotherapy efficacy in models of metastatic ovarian cancer. Our work illustrates a significant and specific contribution of NK cells towards the efficacy of conventional cancer therapy, although local densities of cytotoxic lymphocytes are crucial for this effect. Relatively safe immunotherapies that broadly enhance tissue-resident innate and adaptive immunity therefore represent an attractive target to improve standard cancer therapy.

Brown, Sydney: Ottawa Hospital Research Institute

- *Investigating the neuro-regulation of group 2 innate lymphoid cells in ovarian cancer*

Intro: Ovarian tumors are immunologically "cold" and often have poor responses to immunotherapies. Inducing inflammation in the tumor microenvironment (TME) is one potential strategy to overcome this challenge and improve anti-tumor responses. Indeed, we have shown that treating ovarian tumor-bearing mice with IL-33 and αPD-1 significantly improves survival. This treatment robustly activates ILC2s, and we hypothesize that they are key mediators of this survival benefit. However, we also detected an upregulation of the receptor for the neuropeptide CGRP on IL-33+αPD-1-treated tumor ILC2s. Interestingly, CGRP inhibits proliferation and IL-13 production in lung ILC2s. Although these interactions remain unknown in ovarian tumor ILC2s, upregulation of the CGRP receptor may suggest neuro-immune regulation as a compensatory mechanism for treatment-induced activation. Therefore, we hypothesize that blocking CGRP signaling in ovarian tumors will further improve the ILC2-mediated therapeutic efficacy of IL-33 + αPD-1 treatment.

Methodology: We cultured ILC2s in increasing concentrations of CGRP for 24 hours and analyzed expression of activation markers and effector cytokines by flow cytometry. To assess the effects of CGRP signaling in vivo in our syngeneic orthotopic mouse model of ovarian cancer, we treated mice with PBS (untreated control), Olcegepant (a CGRP receptor antagonist), IL-33+αPD-1, or a combination of all three.

Results: CGRP decreased IL-13 and GM-CSF production and increased amphiregulin production in tumor ILC2s, indicating a shift to impaired anti-tumor functions. Consistent with this, blocking CGRP signaling in vivo increased survival. Olcegepant alone modestly improved survival (median survival 70 days vs 65.5 days for untreated) but was less effective than IL-33+αPD-1 (80 days). However, the IL-33+αPD-1+Olcegepant group survived the longest (87 days), suggesting that Olcegepant further enhances the efficacy of IL-33+αPD-1.

Conclusion: Our findings suggest that CGRP limits the anti-tumor response from ILC2s by altering cytokine production, and that CGRP-blocking drugs could be used to further improve IL-33+αPD-1 therapy.

Cocozza, Germana: Sapienza University of Rome

- *Immune control of sleep pressure via interferon-γ in mice*

Sleep pressure reflects the brain's homeostatic need to sleep, but the mechanisms underlying its regulation remain poorly understood. In mice, a subset of cortical inhibitory neuronal nitric oxide synthase (nNOS) positive interneurons tunes the electroencephalographic slow wave activity in the delta band (<4 Hz), marker of sleep pressure. Here, we demonstrate that in mice the natural killer (NK) cells and innate lymphoid cells (ILC)1 depletion inhibits nNOS+ interneurons and EEG delta activity reducing the time spent in the non-rapid eye movement (NREM) sleep. The optogenetic re-activation of nNOS+ interneurons in the cingulate cortex of NK cell/ILC1-depleted mice rescues the EEG delta activity, confirming the link between innate immune cells-nNOS+ interneurons-sleep pressure. Finally, we demonstrated that meningeal NK/ILC1 cells produceIFN-γ in a circadian independent manner and that IFN-γ blockade in vivo mimics the effect of NK cell depletion in mice. These findings provide insights into the complex network involved in sleep regulation and further support the contribution of the innate immune system on sleep pressure.

Cutter, Alyssa Stanford University

- *CCR6 expression marks a heightened activation state in NKp46+ ILC3s in a mouse strain-dependent manner*

Type 3 innate lymphoid cells (ILC3s) produce cytokines that maintain intestinal epithelial cell homeostasis, initiate lymphoid tissue development, and enhance host defense mechanisms against extracellular pathogens. Here, we report an ILC3 phenotype characterized by co-expression of NKp46 and CCR6, which we observed in multiple inbred and outbred mouse strains but was notably absent in C57BL/6 mice. Balb/c mice were used as a representative model to characterize NKp46+CCR6+ ILC3s. NKp46+CCR6+ ILC3s were present in intestinal lamina propria and were confirmed to be innate lymphocytes, developing in the absence of Rag1 but requiring the common gamma chain receptor subunit. Cultures of purified ILC3 subpopulations demonstrated that NKp46+CCR6+ cells were closely related to NKp46+CCR6- ILC3s, which was further confirmed in vivo by tracking adoptively transferred cells and through cell lineage tracing experiments. NKp46+CCR6+ ILC3s produced more IL-22 on a per cell basis than their NKp46+CCR6- counterparts during both homeostatic and infection conditions, indicating a heightened cell activation state in NKp46+CCR6+ cells. In mice challenged with bacterial flagellin, NKp46+ ILC3s upregulated CCR6 transcription and exhibited changes in tissue localization. This study reveals an additional RORgt+ ILC3 phenotype, uniquely poised to modulate tissue responses, which may be tuned to meet the needs of the intestinal mucosa.

Dalton, Alana King's College London

- *Profiling innate lymphoid cell subsets in paediatric peanut allergy and tolerance*

Food allergy prevalence has increased in recent decades and there is no curative treatment. Understanding immune mechanisms underlying food allergy and tolerance despite the presence of IgE can identify novel therapeutic targets. Innate lymphoid cells (ILC) are thought to regulate allergic responses, with mouse studies linking ILC2 to food allergy through Th2 cytokines and ILC3 to tolerance. Here, we investigate human ILC populations in a paediatric cohort to clarify their role in peanut allergy and tolerance.

Blood samples from a paediatric cohort of peanut allergic (PA) and peanut sensitised tolerant (PST) patients (i.e. peanut-specific IgE and able to eat peanut without symptoms) were analysed by flow cytometry and single-cell RNA sequencing to investigate their ILC populations at baseline and after peanut stimulation. Matched circulatory and duodenal ILCs were also analysed.

Our results show presence of all ILC subsets in both patient groups, with no significant differences in helper ILC subset frequencies between PA and PST in peripheral blood. Stimulation of these subsets with peanut extract increased IL-4 expression by ILC2. Further transcriptional analysis supported the expression of antigen-presenting genes across all ILC populations. Comparison of ILCs from the peripheral blood with ILCs from duodenal tissue of PST patients showed that ILC3 represent the most abundant circulatory subset while ILC1 are predominate in the duodenum. In both ILC1 and ILC3, we observed upregulation of HLA-DR in the intestinal populations.

Together, these findings indicate that ILC subset distributions are comparable between PA and PST children peripheral blood and differ from the ones in the duodenal tissue. Transcriptional signatures observed across ILC subsets, including antigen-presentation–associated genes and increased HLA-DR expression in intestinal ILC1 and ILC3, suggest context-dependent functional specialisation. Further studies are needed to determine how these features contribute to peanut tolerance or allergy in children.

Dey, Prasenjit Roswell Park Cancer Center

- *Innate lymphoid cells drive young-onset lung cancer incidences in females*

Since the recognition of tobacco smoking as a major risk factor for non-small cell lung carcinoma (NSCLC), the rate of incidence in males has significantly declined. However, new cases of lung adenocarcinoma (LUAD), a subset of NSCLC, continue to rise among females and non-smoking populations. Recent epidemiological studies, along with our preliminary analysis from the US National Cancer Database, have found that the incidence of LUAD in females has surpassed that of males in age groups younger than age 50, which suggests factors beyond smoking drive the sexual dimorphism in lung tumorigenesis. There is a growing appreciation for the immune system in various cancers, including LUAD, with increasing evidence of a pro-tumorigenic role of type 2 inflammation. Our comprehensive whole tumor transcriptomic,

scRNA-seq, and immunophenotypic analyses of mouse LUAD tumors have identified multiple immune regulatory pathways, among them type 2 immune cells (innate lymphoid cells 2 [ILC2], TH2, eosinophil, and mast cells) were the top-enriched immune cell types in the TIME of female compared to male mice. ILC2s are the first responders in the host innate immune defense, and recent evidence indicates that ILC2s are the linchpin of type 2 immunity, orchestrating downstream immune responses. Studies have connected sex hormonal signaling to ILC2 regulation that potentially influences female sex disparity in allergic asthma disease, but how ILC2 function is regulated in LUAD remains unknown. Using metabolomic, transcriptomic, and molecular biology analysis, we discovered that female ILC2s have heightened arginine metabolism and are associated with ILC2 function in females. In vitro, analysis of arginine depletion and specific Arg1 inhibition disrupted metabolic programming and function in female ILC2s. These data underscore the role of arginine metabolism in ILC2 function and provide new clues into the low immunotherapy response and decreased survival in females compared to males.

Fiordi, Benedetta: University of Geneva

- *AML blasts shed TREM2 to inhibit group 1 ILCs anti-leukemic function*

Acute Myeloid Leukemia (AML) is characterized by the accumulation of blasts in Bone Marrow (BM) and Peripheral Blood (PB). Group 1 Innate Lymphoid Cells (ILCs including helper ILC1s and Natural Killer cells (NKs)) are present in the BM niche and have anti-leukemic function. However, this ability is lost in AML, where they are IFNγ-deficient. Here we investigate whether the anti-leukemic potential of group 1 ILCs is inhibited by the Triggering Receptor expressed on Myeloid cells 2 (TREM2), given that AML blasts derive from a myeloid lineage and that TREM2 has been shown to impair the anti-tumoral function of NK cells.

We found that TREM2 is expressed preferentially by OCI-AML3, an AML cell line, and that leukemic cells are able to shed the receptor upon ADAM protease activation. In line with this finding, the soluble form of TREM2 (sTREM2) was dramatically increased in PB and BM sera of AML patients at disease onset in comparison to Healthy Donors (HD) and was normalized more than 2 years after hematopoietic stem cell transplantation, a therapeutic option for AML patients, indicating a possible active role in the disease.

Indeed, in the presence of sTREM2, IFNγ secretion by group 1 ILCs in both HD- and AML-derived cells was decreased, confirming its inhibitory role. We identified a potential mechanistic pathway whereby sTREM2 promotes the secretion of IL-18 Binding Protein (IL-18BP) by group 1 ILCs, restricting IL-18 signaling in the same cells and leading to their diminished IFNγ production.In vivo, injection of either patients' cells or the OCI-AML3 cell line in immunodeficient mice recapitulated human sTREM2 increase observed in the patients' serum.

Overall, our data suggest that sTREM2 is increased in AML patients, can be shed upon ADAM protease activation, and inhibits group 1 ILCsIFNγ production via IL-18 sequestration by IL-18BP.

Gong, Hwanseok: Seoul National University

- *Histone Deacetylases Regulate the Persistence and Multipotency of Memory ILC2s in Lung Inflammation*

Type 2 innate lymphoid cells (ILC2s) play a central role in type 2 immune responses and allergic airway inflammation. Recent studies suggest that ILC2s acquire memory-like properties following repeated activation, but the mechanisms governing their persistence and functional regulation remain poorly understood. Here, we demonstrate that memory ILC2s persist long-term in lung tissue and exhibit enhanced recall responses that exacerbate airway inflammation. We further identify histone deacetylases (HDACs) as key regulators for their maintenance and function.

Using murine models of repeated IL-33 stimulation and allergen exposure, we show that memory ILC2s exhibit sustained tissue residency, resistance to apoptosis, and robust secondary expansion upon rechallenge. These cells display a distinct transcriptional program and enhanced multipotency, producing both type 2 and type 17 cytokines. Memory ILC2s also upregulate CD103, which is associated with epithelial interaction and tissue retention.

Transcriptomic analysis revealed enrichment of genes related to epigenetic regulation in memory ILC2s with prominent upregulation of class I HDACs, particularly HDAC1 and HDAC3. Conditional deletion of HDAC1 or HDAC3 in ILC2s significantly impaired memory ILC2 expansion, cytokine production, and airway inflammatory responses without affecting ILC development or primary activation. Mechanistically, HDAC1 and HDAC3 promoted apoptosis resistance, maintained CD103 expression, and supported multipotent cytokine production by memory ILC2s.

Collectively, these findings identify HDAC1- and HDAC3-mediated epigenetic regulation as a critical mechanism controlling memory ILC2 persistence and functional plasticity in lung inflammation. Targeting HDAC-dependent pathways may provide therapeutic opportunities for chronic allergic and inflammatory airway diseases.

Grocott, Sebastian: University of Toronto

- *Development of NK Cell Memory Following Hematopoietic Stem Cell Transplantation*

Natural Killer (NK) cells display features of immunological memory, including clonal expansion, epigenetic diversification, metabolic adaptation, and antigen-specific responses to viruses such as human cytomegalovirus (HCMV). Following hematopoietic stem cell transplantation (HSCT), NKG2C+NK cell expansion is closely linked with HCMV reactivation in seropositive recipients, yet expansions within HCMV-negative recipients has also been reported, highlighting HSCT as

a unique model to explore de novo development of NK cell memory in humans. We performed scRNAseq on a pair of monozygotic twins who received a HSCT >13 years prior and their HLA-matched sibling donor to explore environmental versus genetic influences on NK cell repertoire and function. We identified increased KLRC2(NKG2C)-expressing NK cells in both recipients, who were HCMV-seronegative (IgG-IgM-) upon sampling. Their serostatus diverged from their HSC donor, who seroconverted between donation and sampling. NK cells in recipients exhibited decreased FCER1G and CXXC5 and increased IL32 expression compared to donor NK cells, in line with an adaptive NK cell identity. Inferring clonal architecture through combinatorial KIR gene expression, we observed greater clonotypic diversity in recipients' KLRC2+NK than in donor cells, while KLRC2+NK cells showed greater clonality than conventional NK cells within each NK cell repertoire. Long-term culture of recipient CD16+NK cells demonstrated durable proliferation and enhanced antibody-dependent cellular cytotoxicity compared to the donor. Ongoing studies are characterizing NK cell memory and donor-genotype NK cell repertoires in a cohort of 35 long-term HSCT recipients and their HLA-matched HSC donors (median: 12-years post-HSCT). Pairing multimodal single-cell profiling with functional cytotoxicity assays, and leveraging the variegated expression of germline-encoded KIRs to distinguish oligoclonal subpopulations, we are systematically delineating the distribution of adaptive and memory-like NK cell phenotypes within and across recipient-donor pair repertoires with diverse HCMV serostatus. Collectively, our findings offer insights into innate immune memory development and potential implications for transplant outcomes.

Ibrahim, Dalia: Ottawa Hospital Research Institute

- *Harnessing IL-33 and checkpoint blockade to boost adaptive immunity in ovarian cancer*

Survival rates for ovarian cancer have remained largely unchanged for several decades, in part due to the lack of curative treatments. Therefore, finding new therapeutic strategies is of utmost importance. In exploring possible immunotherapies that modulate the tumor microenvironment, we recently discovered that IL-33 combined with anti–PD-1 improved survival in the ID8 Trp53-/- Brca1-/-syngeneic model of ovarian cancer, and treatment was associated with increased expansion and activity of ILC2s. Based on the known capacity of IL-33 to influence both innate and adaptive immunity, either indirectly through ILC2-mediated crosstalk or directly through effects on T cells, we hypothesized that IL-33 enhances anti–PD-1 efficacy by reshaping adaptive immune responses to promote a less immunosuppressive environment. The overall goals of this project are to define the immune mechanisms underlying IL-33–based combination therapy and to determine whether further checkpoint blockade could enhance therapeutic benefit. Given that IL-33 can expand CTLA-4–expressing regulatory T cells, we evaluated the impact of adding anti–CTLA-4 to IL-33 and anti–PD-1 therapy. While median survival was similar between mice treated with dual therapy (107 days) and triple therapy (117 days), the triple combination

uniquely resulted in 25% of mice remaining tumor-free. Treatment increased the abundance of $CD103^+$ and $CD80^+$ dendritic cells, consistent with enhanced antigen presentation, and was accompanied by heightened activation of $CD4^+$ and $CD8^+$ T cells with increased co-stimulatory signaling, indicating more effective adaptive immune engagement. Treatment also increased B cell abundance in the draining lymph nodes and promoted enhanced tumor-specific antibody production. Importantly, these antibodies were functionally active, promoting antibody-dependent cellular cytotoxicity and providing a mechanistic link between humoral immunity and tumor control. Together, these findings indicate that therapeutic efficacy is driven by coordinated activation of cellular and humoral immunity, informing the rational development of combination immunotherapies for ovarian cancer.

Ito, Mitsuki: RIKEN

- *Acetate-mediated modulation of colonic ILC2s activation by Collinsella aerofaciens shapes IgA-mediated immune homeostasis*

Collinsella aerofaciens (CA), a dominant member of the phylum Actinomycetota, primarily colonizes the human intestinal tract. Ulcerative colitis (UC) is associated with gut dysbiosis, and patients with UC exhibited a marked reduction in CA abundance. However, the immunological role of CA in intestinal immune homeostasis and disease pathology remains largely unclear.

To investigate the immunological role of CA in the large intestine (LI), we generated CA gnotobiotic mice (CA-GB) by introducing a single CA into germ-free mice. Metabolomic analysis revealed increased levels of acetate, a short-chain fatty acid (SCFA) known to mediate immune responses, in the LI of CA-GB. CA colonization enhanced IL-5 production by ILC2s and increased total IgA production. However, CA-specific IgA was barely detectable, suggesting that the CA itself does not directly induce antigen-specific IgA responses. In SPF mice colonized with CA (CA-SPF), luminal total IgA levels remained comparable, whereas the proportion of IgA-coated bacteria was markedly increased. Enhanced IgA coating of commensal bacteria has been reported to strengthen mucosal defense and promote an anti-inflammatory intestinal environment. Consistently, CA-SPF mice exhibited attenuated disease severity in dextran sulfate sodium (DSS)-induced colitis.

These findings indicate that CA promotes ILC2-derived IL-5 production and induces broadly reactive IgA responses that enhance immune regulation of the gut microbiota, thereby contributing to the establishment of an anti-inflammatory colonic environment and amelioration of colitis.

Kagatani Jin: Keio University School of medicine

- *Functional and transcriptional remodeling of peripheral blood ILC2s in asthma*

Background: Type 2 inflammation drives asthma pathogenesis, with airway epithelial–derived cytokines such as IL-33 and TSLP activating group 2 innate lymphoid cells (ILC2s). IL-33/TSLP signaling enhances IL-5 and IL-13 production and has been implicated in steroid resistance. However, functional and transcriptional characterization of circulating human ILC2s remains limited due to their scarcity.

Methods: We analyzed peripheral blood ILC2s (100–500 cells/mL) using live-cell imaging–based single-cell cytokine secretion assays and Smart-seq transcriptomic profiling. ILC2 responses to IL-2+IL-33+TSLP stimulation were compared between healthy controls and asthma patients. In severe asthma, we examined the effects of oral corticosteroids, anti–IL-5 receptor α antibody, and anti-TSLP antibody treatment on ILC2 function and gene expression.

Results: Peripheral blood ILC2s from asthma patients (N = 15) exhibited enhanced IL-5 and IL-13 production upon IL-2+IL-33+TSLP stimulation compared with controls (N = 7). Transcriptomic analysis revealed upregulation of IL4R and downstream signaling pathways, along with increased expression of chemokine-related and proliferation-associated genes, consistent with an inflammatory priming state. In severe asthma (N = 70), maintenance oral corticosteroid use did not significantly alter cytokine production capacity of ILC2s. In contrast, anti–IL-5 receptor α therapy significantly reduced IL-5 and IL-13 production upon stimulation, indicating functional remodeling of circulating ILC2s. Ongoing analyses evaluate the impact of anti-TSLP therapy on cytokine production, steroid responsiveness, and transcriptional reprogramming.

Conclusions: Peripheral blood ILC2s in asthma exhibit enhanced functional responsiveness and distinct transcriptional signatures consistent with inflammatory priming. Biologic therapies differentially reshape ILC2 function in vivo. These findings position circulating ILC2s as functionally plastic immune cells and potential dynamic biomarkers in human asthma.

Kabil, Ahmed University of British Columbia

- *The microbiome and innate lymphoid cells as sculptors of chronic inflammatory disease*

A tightly regulated immune response is essential to ensure protection from the plethora of microbial pathogens we encounter, while simultaneously averting spurious responses to innocuous environmental antigens, self-antigens, or commensal microflora. Advances in Western medicine and sanitation have dramatically reduced mortality and morbidity linked to infectious diseases, but at a cost: diminished microbial exposure has inadvertently altered normal immune cell development and heightened the risk of allergies and autoimmune disorders. Innate lymphoid cells (ILCs) are recently discovered innate counterparts of T lymphocytes but lack antigen specific T cell receptors. Given their early developmental tissue

colonization, longevity, and role as first responders in inflammation, ILCs are ideally positioned as regulators of long-term immune sculpting by the gut microbiome. Employing mouse models of allergic airway inflammation, we demonstrate that perinatal exposure to low doses of vancomycin amplified allergic responses in adulthood. ILC2s emerged as key regulators of peripheral type 2 immune sculpting, driving innate natural IgE production by B1-B cells and increased surface bound IgE on basophils and mast cells, exacerbating allergic inflammation. This enhanced lung ILC2/B1 cell/effector cell axis was completely suppressed with dietary short-chain fatty acid SCFA supplementation. Conversely, streptomycin treatment heightened susceptibility to hypersensitivity pneumonitis, marked by increased lung ILC3s and Th17 cells, and was significantly ameliorated by isolithocholic acid (isoLCA) or mTORC1 inhibition. This highlights that antibiotic-induced dysbiosis, by skewing microbial communities, is remarkably selective in its effects on immune sculpting, with vancomycin exacerbating Th2-disease and streptomycin exacerbating Th1/Th17-linked diseases. We have also generated a novel transgenic mouse strain (Il17rb-CreERT2-eGFP) to selectively target ILC2s. Loss of gastrointestinal ILC2s led to increased ILC3/Th17 responses, and heightened susceptibility to Crohn's disease–like fibrosis. Conversely, dampening ILC3/Th17 responses using isoLCA, a microbial secondary bile acid and RORγt inverse agonist, reduced IL-17 production, protecting against fibrosis.

Kim, Taehyun; University of British Columbia

- *Activated ILC2s enhance intranasal vaccine induced immune memory responses*

Introduction: Effective mucosal vaccination requires the precise orchestration of immune cell trafficking from the site of entry to draining lymph nodes to establish robust systemic memory. Recent advances in intranasal vaccination strategies for SARS-CoV-2 has highlighted the importance of local mechanics in generating protective immunity at the respiratory interface. Group 2 innate lymphoid cells (ILC2s) act as critical sentinels in the lung and have ability to facilitate immune responses through the production of Leukaemia Inhibitory Factor (LIF) that initiates the egress of dendritic cells (DCs) to the mediastinal lymph nodes.

Methods: To evaluate if ILC2 activation can boost vaccine efficacy, we utilized an intranasal model of the Comirnaty Omicron XBB.1.5 SARS-CoV-2 vaccine. Mice were immunized intranasally with the vaccine supplemented with either PBS or the activated form of recombinant IL-33. At day 14 post-immunization, we assessed the mediastinal lymph node for the onset of immune memory and germinal center formation using spectral flow cytometry.

Results: IL-33 adjuvating significantly enhanced the immune response compared to the PBS control. We observed a significant increase in antigen-specific tetramer+ CD8 memory T cells, as well as robust germinal center formations. In addition, we see significantly increased IgG+ and IgM+ memory B cells. Aligning with the enhanced adaptive immunity, we observe more

cDC1s and cDC2s recruited to the mediastinal lymph node. Notably, these effects were only observed in females; males showed no significant difference between groups.

Conclusion & Future Directions: Harnessing the IL-33-ILC2 axis provides a potent strategy for boosting mucosal vaccines by licensing DC migration to draining lymph nodes. We are currently utilizing ILC2-deficient and LIF-neutralization model to establish the mechanistic requirement of ILC2-derived LIF in this response. Additionally, we are investigating the underlying causes of the observed sexual dimorphism in IL-33 responsiveness.

Kinzel, Megan : University of Calgary

Transforming growth factor-beta induces interleukin-22 expression in type 2 innate lymphoid cells and impairs type 2 driven anti-tumour immunity

Introduction: Type 2 innate lymphoid cells (ILC2s) promote type 2-driven anti-tumour immunity, partly through interleukin-5 (IL-5) and granulocyte-macrophage colony stimulating factor (GM-CSF) secretion. In the presence of IL-1β, IL-23, and transforming growth factor-beta (TGF-β), ILC2s can transdifferentiate into ILC3-like cells. We unexpectedly observed in vitro that IL-33-stimulated ILC2s express IL-22 in the absence of IL-1β/IL-23. We therefore investigated whether TGF-β induces a shift in ILC2 identity and how TGF-β affects ILC2-mediated anti-tumour immunity.

Methodology: ILC2s were expanded from the bone marrow-derived ILC2 progenitors of IL-5Cre-TdTomato and IL-5Cre-TdTomatoxTGF-βrIIfl/fl mice. Cells were expanded in IL-2, IL-7, ±IL-25, and ±IL-33 (40 ng/ml) for 28 days. Similarly, lung ILC2s were isolated from C57BL/6 and IL-5Cre-TdTomatoxTGF-βrIIfl/fl mice and cultured ex vivo for 5 days in IL-2, IL-7, IL-33 (40 ng/ml) ±TGF-β (10 ng/ml). IL-22 secretion was assessed via ELISA and ILC2s were phenotyped via flow cytometry. IL-5Cre-TdTomato and IL-5Cre-TdTomatoxTGF-βrIIfl/fl mice were infused with B16F10 melanoma cells to induce lung metastasis.

Results: IL-33-stimulation induced IL-22 production in expanded ILC2s, however this effect was reduced upon TGF-βrIIfl/fl knockout. TGF-βrII knockout maintained ILC2 phenotype via flow cytometry, noted by increased GATA3 and IL-5 expression. Similarly, in C57BL/6 lung ILC2s, TGF-β stimulation induced IL-22 production (ELISA) while reducing GATA3, IL-5, and GM-CSF expression. Mice with TGF-βrII deleted in IL-5+ cells (includes ILC2s) experienced a reduced number of lung metastases 7 and 14 days post-inoculation, associated with increased IL-5 and GM-CSF expression on ILC2s and increased eosinophil recruitment.

Conclusions: These results suggest that TGF-β signalling in ILC2s impairs their type 2 identity and effector function, hindering anti-tumour immunity, while simultaneously inducing ILC2s to secrete IL-22. Whether ILC2-derived IL-22 production further impairs anti-tumour immunity is unclear. These findings demonstrate how local tissue signals can shape and dysregulate ILC2 response, having implications for anti-tumour immunity and type 2 immune responses.

Ko, Young Gyun: Seuol National university

- *GITR-ILC2-IL-9 Axis drives eosinophils-mediated anti-tumor immunity*

Innate lymphoid cells (ILCs) are key regulators of tissue immunity, yet their roles in tumor control incompletely understood. Here, we identify the GITR–ILC2–IL-9–eosinophil axis as a critical pathway mediating anti-tumor immunity. GITR ligation on tumor-infiltrating ILC2s selectively induces ATF3, driving IL-9 production while sparing canonical cytokines such as IL-5 and IL-13. ILC2-derived IL-9 licenses eosinophil cytotoxicity via upregulation of cytotoxic granule genes and tumoricidal activity. This effect is facilitated by tumor microenvironment–induced IL-9 receptor expression on eosinophils. Functionally, IL-9–dependent eosinophil activation suppresses tumor growth in vivo. Analysis of patient datasets revealed IL-9 signatures correlate with favorable prognosis in melanoma and lung adenocarcinoma. These findings delineate a mechanistic pathway linking ILC2s to eosinophil-mediated tumor immunity and nominate GITR as a potential therapeutic target to engage innate lymphoid and granulocyte compartments in cancer.

Marchalot, Anne: Karolinska Institutet

- *Tumor-infiltrating immature innate lymphoid cells in colorectal cancer are biased towards tissue-resident NK cell/ILC1 differentiation*

Approximately 10% of patients with colorectal cancer (CRC) develop peritoneal metastases (PM), often with poor prognosis. While T cells play an undisputed role in CRC, dysregulated ILCs have also been described, but their role in CRC-PM remains unknown. In this translational cohort study including a total of 43 CRC and CRC-PM patients, single-cell RNA sequencing revealed that primary CRC and CRC-PM tumors are infiltrated by heterogeneous populations of ILCs and NK cells that include subsets of ILC3, ILC2, ILC1/tissue resident (tr)NK cells and conventional (c)NK cells. As compared to the unaffected colon, primary CRC and CRC-PM tumors are depleted of ILC3 and enriched for subsets of ILC1/trNK cells and cNK cells. We also identified two subsets of intratumoral immature ILC populations, one identifiable as the previously described naïve (n)ILCs. Intratumoral nILCs showed transcriptional signs of ILC1/trNK cell skewing. Indeed, co-culture with the stromal cell line OP9 overexpressing Delta-like 1 revealed an increased capacity for intratumoral nILCs to generate cells with an ILC1/trNK phenotype, expressing the cytotoxicity molecules perforin and granzyme B, while in parallel expressing tissue residency markers CD49a and CD103. These cells lacked CD16, indicating that intratumoral nILCs are not generating fully mature NK cells. Hence, the data shows that intratumoral nILCs can undergo local differentiation to intratumoral ILC1/trNK cells, possibly contributing to the accumulation of these cells seen in both primary CRC and CRC-PM tumors. These findings contribute to the understanding of the immune pathogenesis of CRC-PM and our large single cell data provide an unprecedented wealth of insight to be used for rational design of future ILC1/NK cell-based therapies aimed at increasing their anti-tumor activity.

Mathews, Jessica Institut Pasteur

- *Investigating a three-signal model for human ILC precursor development*

Innate lymphoid cells (ILCs) have a key role in early immune responses during infections, inflammation, stress, and tissue repair. Three ILC groups have been described in mouse and human, known as ILC1, ILC2, and ILC3 which can be considered as innate homologues of T helper (Th) Th1, Th2, and Th17/22 due to their striking similarities of transcription factors and cytokine production. However, our understanding the regulatory mechanisms guiding human ILC maturation remains incomplete. Circulating 'naive' ILC precusors (ILCP) poised for further differentiation may generate mature ILC in inflamed tissues 'on demand'. Here we explore the signals that dictate primary cell fate decisions in human ILC precursors to uncover the pathways driving specific ILC differentiation. Using the '3-signal' model for Th differentiation, we investigate the impact and role for activating receptors, co-stimulatory molecules and polarizing cytokines on ILC expansion and differentiation. Our results demonstrate that co-stimulatory molecules support ILC2 differentiation, potentially via intracellular IL-2 expression. Our modular in vitro ILCP differentiation system should deepen our understanding of this complex process and may ultimately provide a basis to design methods to direct ILC differentiation that may find translational applications.

Mazzarella, Letizia: Sapienza Università di Roma

- *Natural Killer cells modulate peri-tumoral neuronal activity in glioblastoma*

Natural Killer (NK) cells are key effectors of innate immunity, yet their role in the pathological brain remains poorly defined. In glioblastoma (GBM), an immunosuppressive tumor, NK cell infiltration is limited and their cytotoxic activity is attenuated. In parallel, peri-tumoral neuronal circuits undergo extensive remodelling, shaping the balance excitation/inhibition with effects on GBM-related epilepsy.

Here, we investigated the involvement of NK cells in modulating neuronal fate and activity in murine GBM models. First, in vitro assays showed increased recruitment of NK cells to neuronal neurites in GBM-conditioned media and NK cell-mediated cytotoxicity on neuronal soma, whereas in GBM-bearing mice NK cells were detected at synaptic sites of peri-tumoral neurons, where they contribute to the modulation of synaptic density.

This structural interaction is linked to functional changes in neuronal excitability, with whole-cell patch-clamp recordings revealing increased firing of excitatory peri-tumoral neurons after NK cell depletion in GBM-bearing mice, suggesting a potential contribution to epilepsy.

Spatial transcriptomic analyses further indicated that NK cells display distinct transcriptional programs depending on their location in the tumor core versus peri-tumoral regions, suggesting regional functional specialization. Although the precise mechanisms of NK cell–

neuron interactions remain under investigation, these findings point to a direct structural and molecular interface, potentially mediated by activating NK cell receptors.

Together, our results uncover an unexpected role for NK cells in regulating peri-tumoral neuronal circuits in GBM, highlighting innate immunity as an active contributor to the modulation of neuronal activity in the diseased brain.

Mormino, Alessandro: Sapienza Università di Roma

- *Natural killer cells prime microglial synaptic pruning necessary for physiological memory formation through the release of interferon-gamma.*

Proper microglial activity is necessary for synaptic pruning and the establishment of memory. To exert its function, microglia receive a wide plethora of molecular signals that are poorly understood. It is known that microglia are sensitive to interferon-gamma. To evaluate the possible role of the cytokine on microglial cells, we first specifically suppressed the interferon-gamma receptor 1 (IFN-γR1) expression in microglia using a dendrimer-conjugated siRNA. This manipulation led to a deregulation of genes involved in synaptic pruning, impaired performance in spatial memory tasks, and increased density of dendritic spines with an increased percentage of immature spines. Since STAT1 is a critical downstream effector of IFN-γ signaling, we confirmed the effects of the silencing, using STAT1 knockout mice that showed behavioral, morphological, and transcriptional alterations similar to those upon microglial IFN-γR1 silencing, proving the functional relevance of this pathway. Similarly, data obtained by mice treated with XMG1.2, an interferon-gamma blocking antibody, confirmed the essential role of IFN-γ in the regulation of microglial activity. Finally, to assess the possible source of IFN- γ in the central nervous system, we depleted NK cell from mice. Microglia from NK-depleted mice expressed greater levels of inflammatory genes, reduced pruning-related gene expression, more ramified morphology, and fewer microglia–neuron contacts, resulting in impaired memory performance. Collectively, these data support the existence of a NK cell-microglia communication axis that is pivotal for the physiological microglial function.

Nachataya, Katie: Amsterdam UMC

- *Mapping IL-23 Target Cells and Downstream Pathways in Inflammatory Bowel Disease*

IL-23 is a key cytokine implicated in intestinal inflammation and is a major therapeutic target in inflammatory bowel disease (IBD). Aberrant IL-23 signalling sustains pathogenic immune responses, yet the cellular targets and mechanisms through which IL-23 perpetuates tissue damage remain incompletely understood.

To dissect IL-23 responsiveness in the human intestine, we first analyzed existing single-cell RNA sequencing datasets, identifying innate lymphoid cells type 3 (ILC3s) and mucosal-associated invariant T (MAIT) cells as the main IL-23R-expressing populations. Guided by these findings, we conducted a first-of-its-kind longitudinal in vivo study,in which biopsies from Crohn's disease patients were collected before and after treatment with IL-23A- and IL-12B-blocking antibodies, risankizumab and ustekinumab, respectively. To enable detailed characterization of IL-23-responsive pathways within the intestinal microenvironment, biopsies were enriched for ILC3s and MAIT cells by flow cytometry before single-cell RNA sequencing. This targeted enrichment markedly increased the representation of ILCs—yielding a 20-fold higher abundance compared to whole-biopsy sequencing—while preserving the full diversity of cellular populations present in the tissue. As a result, we achieved substantially improved resolution of ILC populations and were able to more clearly define their phenotypic states and interactions within the intestine.

Initial analysis revealed that ILC3s in inflamed intestinal tissue before IL-12 and IL-23 blockade exhibit elevated expression ofGNLY,TNFSF4, andITGAX,consistent with their established involvement in IBD-associated inflammation. Moreover, this approach uncovered an expanded set of IL-23–regulated genes in ILCs in vivo, includingVIDR,BATF,FURIN,andZNRF1, further defining the transcriptional programs driven by IL-23 in the human intestinal immune compartment. Ongoing work aims to further dissect the role of IL-23–regulated effectors in intestinal inflammation using organoid–ILC co-culture systems.

Naef, Pascal: University of California, Irvine

- *Type 2 innate lymphoid cells exert context-dependent functions in breast cancer*

The breast cancer (BC) tumor microenvironment (TME) contains immune populations that can either restrain or promote disease. Type 2 innate lymphoid cells (ILC2s) are enriched at barrier tissues, yet their role in mammary tumorigenesis remains unclear. Here, we define how ILC2s shape the BC TME and evaluate their therapeutic potential.

We crossed the polyoma middle T antigen (PyMT) transgenic model of luminal BC with ILC2-deficient mice. ILC2 deficiency delayed tumor onset and prolonged time to endpoint, indicating a tumor-promoting role for endogenous ILC2s during spontaneous mammary carcinogenesis. Consistently, orthotopic co-injection of in vitro-expanded bone marrow ILC2s with the VO BC cell line increased tumor take and tumor size in FVB recipients and enhanced infiltration of myeloid-derived suppressor cells (MDSCs), while co-culture with mammary fat pad (MFP) ILC2s increased BC cell line survival under nutrient deprivation. Taken together, these results suggest that ILC2s promote an immunosuppressive niche and engage in direct protective crosstalk with tumor cells. Single-cell RNA sequencing and flow cytometry revealed progressive accumulation of canonical ILC2s (expressing Gata3, Rora, Il2ra, Il7ra) in the MFP

during tumor development, accompanied by phenotypic remodeling with increased PD-1 and Neuropilin-1 expression.

Given this acquired PD-1 expression, we asked whether ILC2 hyperactivation combined with PD-1 blockade may reverse ILC2 pro-tumor activity. Overactivation of the ILC2s with exogenous IL-33 in Py8119 BC tumor-bearing mice reduced tumor burden and enhanced marked infiltration of PD-1+ ILC2s and eosinophils. ILC2-specific PD-1 knockdown further reduced tumor size, confirming that PD-1 acts as a checkpoint that restrains anti-tumor activity in hyperactivated ILC2s.

Together, our data show that ILC2s promote BC progression under baseline conditions yet can be reprogrammed toward tumor control through an IL-33–driven, PD-1–limited activation state, highlighting an actionable ILC2 checkpoint axis.

Okeefe, Ryan: Olivia Newton-John Cancer

- *Circulating inflammatory ILC2s increase with gastric disease progression and share features with IL25 conditioned intestinal ILC2s*

Innate lymphoid cell 2 (ILC2) responses are regulated by epithelial cytokines and expand during type 2 immune activation, but how circulating inflammatory ILC2s (iILC2s) change during gastric disease is not well defined. We quantified blood iILC2s by flow cytometry in murine models spanning gastric metaplasia, early tumour development, and late-stage disease, alongside serum IL25, IL33, and IL13. Functional identity was assessed by ex vivo stimulation with IL25 or IL33 and measurement of IL5, IL13, and amphiregulin, together with phenotypic features linked to plasticity and trafficking. To test mechanistic linkage to the tuft cell–ILC2 axis, tuft cells were ablated across disease settings and ILC2 abundance was assessed in blood and gastric tissue. In gp130F/F tumour-bearing mice, therapeutic targeting of the circuit was used to evaluate effects on iILC2 appearance in blood, and specificity was assessed using a non-gastric tumour model. Finally, we analysed circulating ILC2s in blood from gastric cancer patients and healthy controls, including IL17RB+ and ST2+ subsets, other ILC lineages, and checkpoint marker expression.

Blood iILC2s increased across murine gastric disease stages with accompanying changes in serum cytokines. ILC2s produced type 2 mediators following IL25 or IL33 stimulation, with disease-associated phenotypic differences. Tuft cell ablation reduced blood iILC2s and altered gastric tissue ILC2 abundance, and therapeutic targeting of the circuit reduced iILC2 appearance in tumour-bearing mice. In contrast, circulating ILC2s did not increase in an intrasplenic PDAC model. In humans, circulating ILC2s were higher in gastric cancer than healthy controls, with subset-level differences and reduced PD-1 expression in cancer, while CTLA-4 showed no consistent change. Together, these data link increases in blood iILC2s to gastric disease and tuft cell–ILC2 circuit activity across mouse models and human samples.

Orangi, Mona: University of British Columbia

- *ILC1 and ILC2 promote diet- and alcohol-induced steatohepatitis and fibrosis*

Alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) can advance to steatohepatitis and fibrosis. Group 1 innate lymphoid cells (ILC1s) have been implicated as pro-inflammatory effectors in ischemia–reperfusion injury in fatty livers. ILC2s have also been linked to hepatic fibrosis in ConA-induced hepatitis and CCl_4-driven fibrosis models. However, how ILC populations contribute to ALD and NAFLD progression remains poorly defined.

We established a mouse model of chronic steatohepatitis and fibrosis by administering alcohol together with a high-fat diet to B6 mice to mimic human disease. Using mutant strains lacking specific lymphocyte populations, we assessed steatohepatitis and fibrosis by flow cytometry, histopathology, single-cell RNA-seq, and spatial transcriptomics to identify the immune populations driving disease.

The alcohol–high-fat diet induced hepatitis by 6 weeks and fibrosis by 9 weeks. RAG1-deficient mice developed both steatohepatitis and fibrosis, supporting a minimal role for adaptive immunity. In contrast, NSG mice and ILC-deficient conditional knockout mice (generated by crossing Rora-IRES-Cre with Il7rflox)) did not show a significant increase in hepatic myeloid cells, consistent with a requirement for ILCs in hepatitis. RORγt-KO mice were unaffected, ruling out ILC3s. Single-cell RNA-seq identified three liver ILC1 clusters, with preferential expansion of a cluster 3 ILC1 population following treatment; this expansion was reduced in ILC-deficient mice. Neutrophil increases were also lower after $NK1.1^+$ cell depletion, suggesting ILC1 involvement in hepatitis. Spatial transcriptomics revealed co-localization of ILC1 and Kupffer cell, suggesting that ILC1–Kupffer cell interactions may initiate hepatitis. Reduced Tgfb1+ macrophages at 6 weeks and decreased collagen deposition at 9 weeks in ILC-deficient mice compared with WT controls suggest that ILC2-driven type 2 immunity promotes fibrogenesis. Together, these data indicate that ILC1s and ILC2s are key contributors to alcohol–high-fat diet–induced chronic steatohepatitis and fibrosis.

Papapetropoulos, Chloé: Institut Pasteur

- *Patrolling ILC3s orchestrate antitumor immunity in intestinal cancer*

Group 3 innate lymphoid cells (ILC3s) are tissue-resident immune cells which have emerged as orchestrators of lymphoid development, inflammation, and intestinal immune defense. Although their functions in mucosal homeostasis and immunity have been extensively studied, their contribution in tumorigenesis remains poorly understood. Here, we explore the potential role of intestinal ILC3s in cancer development and progression using and orthotopic mouse model of intestinal carcinoma. We found that CD49a+ ILC3s infiltrate tumors at the early stages of tumorigenesis. The tumor microenvironment signals affect ILC3 behavior and induce a tumor-specific patrolling phenotype along the tumor vasculature that is associated with increased production of interleukin-22 and granulocyte-macrophage-colony-stimulating factor.

Within the tumor, intestinal ILC3s sustain an inflammatory phenotype in tumor-associated macrophages, thereby contributing to anti-tumor immunity. Collectively, our findings identify CD49a+ ILC3s as early tumor-infiltrating immune sentinels that actively contribute to anti-tumor response.

Pinero Garasa, Maria Angeles: Karolinska Institutet

- *Dynamics of ILC2s in the progression of bone marrow fibrosis*

Hematological cancers of the myeloid lineage originate in the BM and may progress to fibrosis in late disease stages. Type 2 inflammation, driven by IL-4 and IL-13, has recently been shown to promote BM fibrosis (BMF). While T cells and mast cells may be implicated, the role of ILC2s in shaping the fibrotic response remains unclear.

We aim to investigate the role of ILC2s in BMF using a mouse model of the myeloproliferative neoplasms (MPN). Inducing mutant thrombopoietin receptor genes (MPL model) drives myeloid hyperproliferation, mirroring the clinical features of the human disease.

In the early disease stages, we observed a reduction of KLRG1+ ILC2 in the BM and concomitant increase in circulation, suggesting local activation and egress from the BM. As disease progressed, CD25+ ILC2s accumulate in the spleen. At fibrosis onset, BM ILC2s exhibited markedly elevated expression of the inhibitory receptor PD-1. Fibrotic mice present extramedullary hematopoiesis and develop spleen fibrosis with concomitant accumulation of PD-1^{+} ILC2s. In contrast, although splenic T cell subsets also expressed PD-1, they did not display a comparable accumulation in the tissue.

In line with our findings, preliminary analysis of peripheral blood from patients in early stages of the disease, showed a shift towards an ILC2 phenotype within the ILC compartment.

Together, our findings suggest a stage specific involvement of ILC2s in the progression of BMF. Our work may uncover a novel function for BM-ILC2s, positioning them as a critical player within the immune landscape driving the progression of myeloid malignancies to bone marrow fibrosis.

Roth, Frederik: McGill University

- *Cholesterol metabolism drives effector functions and maintains metabolic fitness of ILC2*

Group 2 innate lymphoid cells (ILC2s) are key drivers of asthma through rapid and potent secretion of type 2 cytokines. Further, ILC2s mediate allergic inflammation and tissue repair by influencing local and systemic metabolism. In addition to fatty acids having been described as

important fuel for pathogenic ILC2 functions, recent metabolomic studies have associated altered cholesterol metabolism with disease pathogenesis in asthmatic patients. However, the impact of the bioavailability of key metabolites in the tissue microenvironment as well as which metabolic pathways are active in ILC2s remains an underexplored aspect of ILC2 biology that may provide insights to define novel therapeutic targets to treat type 2 immunopathologies. Here we demonstrate that ILC2s upon activation increase exogenous cholesterol uptake, de novo cholesterol synthesis, as well as steroid production and release. In particular, the steroid pregnenolone, synthesized via the steroidogenesis rate-limiting enzyme cytochrome P450 11A1 (CYP11A1), was produced and secreted at high levels by activated ILC2s. Utilizing high-resolution microscopy of primary ILC2s, we further reveal the redistribution of intracellular cholesterol in activated ILC2s. Cholesterol synthesis and steroidogenesis were found to maintain cellular and mitochondrial fitness and facilitate the metabolic flexibility of ILC2s, allowing for the usage of either glycolysis or oxidative phosphorylation as a means of energy and metabolite production in a context-dependent manner. Moreover, we studied the effects of impaired cholesterol synthesis and steroidogenesis in ILC2s by targeting HMG-CoA reductase (HMGCR), the rate-limiting enzyme of cholesterol synthesis and common target of statins, as well as CYP11A1, respectively. The lack of functional cholesterol synthesis or steroidogenesis in ILC2s led to limited promotion of allergic airway inflammation in mouse models of asthma. Collectively, our findings reveal that uptake, synthesis, and catabolism of cholesterol allows maintained mitochondrial fitness to drive the effector functions of ILC2, highlighting HMGCR and CYP11A1 as promising pharmacological targets to counter detrimental type 2 immunopathologies.

Roy-Dorval, Audrey: McGill University

- *GPR18, a Novel Regulator of ILC2 and Allergic Airway Inflammation*

Group 2 innate lymphoid cells (ILC2), play an important role in type 2 immunopathologies through the secretion of type 2 cytokines and the exacerbation of antigen-specific Th2-mediated immunity. A deeper understanding of how ILC2 functions can be regulated is therefore critical for developing novel pharmaceutical strategies to counteract type 2 immunopathologies, including allergic airway inflammation. ILC2 are critical in shaping tissues and whole-body metabolism. In turn, ILC2 are regulated by metabolic as well as neuroendocrine cues, although the mechanistic underpinnings are ill-defined. As select G protein-coupled receptors (GPCRs) were found to shape ILC2 mechanists, we reasoned that metabolic cues utilize GPCRs to regulate ILC2 effector functions and systematically analyzed GPCR expression patterns of ILC2. Here we demonstrate that human as well as murine ILC2 from distinct tissues, including the lungs, express GPR18, which has been suggested to act as a receptor for select diet-derived lipid metabolites. Although already present at low levels at steady state, activation of ILC2 led to a marked increase of GPR18 expression. Ligation of GPR18 with newly developed pharmacological ligands demonstrated that activation of GPR18-

signaling blunted ILC2 proliferation, cytokine release as well as allergic airway inflammation. Mechanistically, we further show that GPR18 ligation altered mitochondrial respiration and ILC2 cell cycle.In conclusion, GPR18 is a potent negative regulator of ILC2 effector functions and allergic airway inflammation through modulation of mitochondrial activity and cellular metabolism.

Sadeghalvad, Mona: BC Cancer Research Centre

- *CD3ε⁺ ILC3s: A Thymus-Dependent Subset Exhibiting Enhanced Cytokine Responsiveness*

CD3 expression has long been considered a hallmark of T-lineage cells, used universally to define and identify T cells. Here, we identify a population of lung innate lymphoid cells (ILC3s) that unexpectedly expresses surface CD3ε while lacking T cell receptor expression. ILC3s are a rare population in the mouse lung but contribute to pulmonary inflammation through IL-17A production in response to inflammatory cytokines such as IL-1β and IL-23. Despite their functional importance, lung ILC3s remain poorly characterized due to their scarcity.

In single-cell RNA sequencing datasets, CD3ε⁺ ILC3s were transcriptionally indistinguishable from T cells. However, flow cytometric analysis revealed their presence in Rag1$^{-/-}$ mice, demonstrating that they are not aberrant T cells that have downregulated the T cell receptor. Because surface CD3ε is widely considered a definitive T cell marker, this population has likely been excluded from prior ILC3 analyses. Although CD3ε⁺ ILC3s represent a minor subset in naïve lungs, they rapidly expand following intranasal IL-1β administration and become the dominant IL-17A–producing population. Notably, CD3ε⁺ ILC3s were absent in nude mice but enriched in the thymus, supporting a thymic origin. Together, our findings uncover a thymus-derived, CD3ε-expressing ILC3 subset with potent inflammatory capacity, revealing an overlooked contributor to pulmonary inflammation

Sasahara, Kotaro: Keio University School of medicine

- *Lentiviral reprogramming of ILC2s generates FOXP3-independent IL-10–producing regulatory ILCs*

Background: Although regulatory T cells (Tregs) are defined by FOXP3 expression and IL-10 production, a FOXP3-dependent regulatory program within innate lymphoid cells (ILCs) has not been fully established. While IL-10–producing ILC subsets have been described, strategies to generate durable regulatory ILCs with therapeutic potential remain limited. Given that ILCs lack antigen-specific receptors and respond directly to cytokine cues, engineered regulatory ILCs may enable antigen-independent immunomodulation.

Methods: We developed a lentiviral transduction platform enabling efficient gene delivery into murine and human ILC2s (Irie M, Kabata H, et al. Cell Rep, 2023; Irie M, Kabata H, et al. STAR Protoc, 2024). Using this system, we induced transcriptional reprogramming of ILC2s to generate IL-10–producing regulatory ILCs (induced ILCreg). Cytokine production, lineage marker expression, and functional activity were evaluated in vitro and in vivo using Alternaria- and LPS-induced airway inflammation models.

Results: Lentiviral-mediated genetic modification of murine ILC2s induced robust IL-10 production without induction of FOXP3 expression, indicating acquisition of a FOXP3-independent regulatory phenotype. Engineered cells exhibited suppression of canonical type 2 cytokines, including IL-5 and IL-13. Adoptive transfer of induced ILCreg significantly attenuated airway inflammation in both Alternaria- and LPS-induced models. Importantly, this platform was successfully translated to human ILC2s; gene-modified cells derived from five independent donors consistently demonstrated enhanced IL-10 production and reduced type 2 cytokine output.

Conclusions: We establish a gene engineering platform that enables stable induction of FOXP3-independent IL-10–producing regulatory ILCs. This strategy highlights the plasticity of ILC2s and provides a proof-of-concept framework for antigen-independent cell-based immunotherapy.

Stadler, Tomasz: Karolinska Intitutet

- *Multidimensional characterization of tissue-specific human innate lymphoid cells across tissues*

Innate lymphoid cells (ILCs) are the innate counterparts of T lymphocytes and are classified into type 1 (NK cells and ILC1s), type 2 (ILC2s), and type 3 (ILC3s and lymphoid tissue inducers cells) subsets based on shared transcriptional programs and effector functions. Despite increasing insight into ILC biology, tissue-specific heterogeneity of human ILCs remains poorly explored. Here, we profiled ILCs across matched tissues from human organ donors to identify tissue-specific signatures with potential functional relevance.

We characterized human CD127+ILCs and CD56+NK cells across matched mucosal and non-mucosal tissues from organ donors (n=10) using a 36-color spectral flow cytometry panel with subsequent multidimensional analysis. Cryopreserved lymphocytes from appendix, ileum, cecum, colon, mesenteric lymph nodes (MLN), lung, liver, spleen, and blood were analyzed and compared with resident T-cell subsets. We identified ILC2s (CRTH2+), ILC3s (NKp44+/-), naïve ILCs (CD45RA+) and NK subsets: early CD16-CD94+/NKp80-(Stage 3-4a), intermediate CD16-NKp80+(Stage 4b), and mature CD16+(Stage 5-6). ILCs (CD45+Lin-CD19-CD3-CD94-CD127+cKit+/-CD161+) were enriched in gut tissues, while NK cells (CD45+LIN-CD19-CD3-CD94+/-CD56+) dominated in non-gut tissues. ILC3s (NKp44+/-) were enriched in all tissues

but lung and blood, that showed higher ILC2 (CRTH2+), naïve ILC (CD45RA+) frequencies. NK cells exhibited site-specific maturation, with early-mid stages enriched in gut tissues and mature subsets in lung, liver, spleen, and blood.

ILCs and NK cells were analyzed with respect to expression of the well-defined T-cell residency markers CD69, CD49a and CD103. CD69+NKp44+/- ILC3s were consistently enriched in lymphoid tissues (MLN and spleen) and gut tissues rich in lymphoid structures (cecum and appendix) compared to colon, ileum, lung, and blood. Lung NKp44+/- ILC3s exhibited tissue-unique CD103 expression. Gut and lung NK cells showed a prominent CD69+/- CD49a+CD103+ phenotype compared to liver, spleen, and blood.

Our study maps the heterogeneity and tissue-specific distribution of human ILCs and NK cells, providing insights into their potential roles in health and disease.

Stehle, Christina: Charité - Universiätsmedizin Berlin, Institute of Medical Immunology

- *Regulation of ILC differentiation and maintenance by the intestinal epithelium*

Daily confronted with microbiota, pathogens and nutrients, the intestine harbours high numbers of tissue-resident innate lymphoid cell (ILC) subsets, with the small intestinal lamina propria representing the major residence site of mature ILC3. While the fetal liver and the adult bone marrow have been proposed as the main sites of ILC development pre- and postnatally, respectively, increasing evidence indicates that ILC differentiation may additionally occur in peripheral tissues. Accordingly, we show that the fetal gut harbours not only mature ILC subsets but also immature populations such as common lymphoid and ILC-committed progenitors, strengthening the concept that the fetal intestine may represent a site of ILC-poiesis during ontogeny. Based on this data, we aimed at characterizing the niche and relevant cytokine sources promoting ILC differentiation in the intestine and show that the intestinal epithelium contributes to IL-7 production for intestinal ILC3 differentiation and maintenance postnatally

Weekers, Jochem: Erasmus Medical Centre

- *The gatekeeper between two kingdoms: ILC2s connect the nervous and immune systems to orchestrate inflammation in asthma.*

Objective: Group 2 innate lymphoid cells (ILC2s) localize near peripheral nerves and pulmonary neuroendocrine cells (PNECs). Interactions between these cells have been shown to modulate inflammation and tissue homeostasis in animal models. In asthma, a chronic pulmonary disease in which CD45RO+ ILC2s are key drivers, patients show increased innervation of the lung and PNEC hyperplasia, suggesting that increased neuro-ILC2 crosstalk may be involved in exacerbating chronic inflammatory disease. However, the extent and exact nature of such cellular communication in human cells remains poorly understood. Here, we explored the

effect of soluble mediators of neuro-ILC2 crosstalk on the phenotype of human tissue-resident-like CD45RO+ inflammatory ILC2s and sensory neurons.

Methods: CD45RA+ ILC2s were isolated from peripheral blood mononuclear cells of healthy individuals and differentiated into CD45RO+ ILC2s, which display a tissue resident phenotype, by exposure to alarmins (IL33/TSLP) in vitro. After 7 days, conditioned medium was collected and used to establish the effects of ILC2-derived factors on induced pluripotent stem cell (iPSC)-derived sensory neuron cultures via immunofluorescence microscopy. CD45RO+ ILC2s were treated with the neuromedin U (NMU) and vasoactive intestinal peptide (VIP), and phenotypic changes were analyzed by RNA-sequencing and flow-cytometry.

Results :In inflammatory ILC2s, transcriptome analysis revealed that neuropeptides regulate a myriad of processes related to lymphocyte function. This included upregulation of cell proliferation genes and downregulating pro-apoptotic genes. Moreover, ILC2s stimulated with neuropeptides showed increased expression of various signaling receptors, chemokines related to tissue homing of asthma-associated immune cells, and altered type 2 cytokine production capacities. Sensory neurons also showed responsiveness to ILC2-derived factors when exposed to conditioned medium, exhibiting increased survival and plasticity.

Conclusion: These results reveal that human ILC2s and sensory neurons have the capacity to engage in extensive communication, acting as a potential bridge between the nervous and immune system. Our data further supports a potential role for neuro-ILC2 crosstalk in asthma pathophysiology.

Wu, Christine: Columbia University

- *METTL3 divergently regulates ILC2 and TH2 cell effector function in allergic asthma*

Allergic asthma is the most prevalent form of asthma and is dominated by the type 2 immune response. Group 2 innate lymphoid cells (ILC2s) and allergen-specific T helper 2 (TH2) cells promote airway inflammation through the production of cytokines, such as interleukin (IL)-4, IL-5, and IL-13.Dynamic changes occur in the transcriptome of ILC2s and TH2 cells following stimulation of alarmin cytokines or allergens, respectively, to prompt activation and proliferation. The most abundant post-transcriptional modification in the eukaryotic transcriptome, N6-methyladenosine (m6A), regulates RNA stability, splicing, translation, and structure. Previously, our lab found that deletion of METTL3, the methyltransferase unit of METTL3-METTL14-WTAP 'writer' complex that installs m6A on mRNAs, abolished IL-25-induced ILC2 proliferation and cytokine production during helminth infection. In this study, we sought to explore how m6A deposition by METTL3 regulates ILC2s and TH2 cell effector response in a house dust mite (HDM) model of allergic asthma. In a mouse model of HDM-induced allergic asthma, ILC2s that lacked METTL3 had perturbed expression of identity transcription factor GATA3. Furthermore, these ILC2s had reduced IL-5 and IL-13 production,

and infiltration of eosinophils in the bronchioalveolar lavage fluid (BALF) was diminished. In contrast, depletion of METTL3 in CD4+ T cells promoted TH2 cell differentiation at steady state. We observed an increase in IL-5 and IL-13 production in the BALF and a concomitant exacerbation of BALF eosinophilia. Our findings highlight that despite their overlapping cytokine profiles and effector functions, ILC2s and TH2 cells possess divergent mechanisms for regulating the transcriptome via m6A methylation.

Xue, Jingna: University of Calgary

- *Spatial Characterization of Innate Lymphoid Cells in the Human Breast Cancer Tumor Microenvironment*

Innate lymphoid cells (ILCs) are tissue-resident immune populations that regulate inflammation, tissue remodeling, and anti-tumor immunity. Among them, group 2 ILCs (ILC2s) have been implicated in both pro- and anti-tumor functions in different types of cancer. Our preliminary data in mouse models showed that ILC2s infiltrate the tumor and promote mammary cancer development, suggesting a pro-tumorigenic role.

Therefore, to consolidate this finding, we performed ultra-multiplexed spatial imaging on a human breast cancer tissue microarray (TMA) using the MACSima platform (Miltenyi Biotech®). We designed and optimized a 45-plex panel, which contains markers to identify multiple T cells (e.g., CD4, CD8, Tregs), B cells, plasma cells, Macrophages, DCs, NKs, and ILC2s (CD45+CD3-GATA3+). Antibodies were selected and validated on FFPE human tonsil control samples, and staining conditions (i.e., concentration, incubation time, and laser power) were optimized to minimize background and maintain signal stability. Cell segmentation will then be performed, and cell proportion, distribution, and cell-cell proximity will be analyzed using the developed pipelines. Lastly, survival outcomes will be analyzed using multivariable Cox proportional hazards regression incorporating recognized prognostic covariates, including sex, age at diagnosis, tumor size, tumor grade, nuclear grade, ER and PR status, and the Oncotype DX recurrence score.

This study provides a spatially resolved characterization of ILC2s in human breast cancer and tests whether their abundance and organization associate with clinical outcomes. These findings may identify ILC2s as a previously underrecognized immune component with potential prognostic and therapeutic relevance.

Yashiro, Takuya University of Osaka

- *Proprotein convertase FURIN is involved in the effector function of group 2 innate lymphoid cells*

Interleukin-33 (IL-33) is a key alarmin that drives ILC2 proliferation and type 2 cytokine production, in part through activation of the p38 MAPK pathway and the master transcription

factor GATA3; however, the molecular mechanisms that couple IL-33 signaling to ILC2 expansion and effector function remain incompletely defined.

To identify novel intracellular regulators of IL-33–mediated ILC2 activation, we performed an integrated analysis of single-cell and bulk RNA sequencing datasets and found that ILC2s express high levels of Pcsk3/Furin, a proprotein convertase that processes precursor proteins into their bioactive forms.Pharmacological inhibition or siRNA-mediated knockdown of FURIN did not affect IL-33R expression or p38 phosphorylation, indicating intact proximal IL-33 signaling, but significantly impaired IL-33–induced ILC2 proliferation and type 2 cytokine production. Notably, FURIN inhibition led to G1 arrest in the cell cycle and reduced the protein levels of type 2 cytokines without altering their mRNA levels. To further examine the role of FURIN in vivo, we generated Il7raCre/+Furinfl/fl mice, in which Furin is selectively deleted in T cells and ILCs, and induced airway inflammation by intratracheal IL-33 administration. Consistent with the in vitro findings, ILC2 proliferation and type 2 cytokine production were significantly reduced in Il7raCre/+Furinfl/fl mice. Similarly, pharmacological inhibition of FURIN in wild-type mice also attenuated IL-33-induced airway inflammation.

In summary, our study uncovers FURIN as a critical mediator that couples IL-33 signaling to ILC2 proliferation and effector function, thereby providing mechanistic insight into type 2 immunity and highlighting FURIN as a promising target for therapeutic intervention.

Yip, Thomas University of Cambridge

- *ILC2 regulate a Pi16+ fibroblast niche with progenitor function under homeostatic, inflammatory, and neoplastic conditions in the pancreas.*

Background:Group 2 Innate Lymphoid Cells (ILC2s) are tissue-resident innate immune cells critical in orchestrating type-2 immune responses, and reside in mesenchymal niches in close contact with fibroblasts. Recent studies have described a pan-organ hierarchical organisation of the fibroblast lineage. Here, we profiled ILC2s of the exocrine pancreas, and investigated ILC2-fibroblast crosstalk in homeostasis, acute pancreatic inflammation, and cancer.
Methods: Naïve, inflamed, and neoplastic pancreata were profiled by flow cytometry, histology, or multiplex immunofluorescence imaging. We assessed two-way communication between ILC2s and fibroblasts usingin silicoanalysis of single-cell and bulk transcriptomic data. Putative crosstalk mechanisms were tested within vitroandin vivoassays.

Results: ILC2 numbers were strongly correlated with fibroblast abundance in homeostasis, and imaging studies showed that most ILC2s co-localise within the pancreatic interstitial niche co-inhabited by Il33+fibroblasts and Lyve1+tissue-resident macrophages. Single-cell transcriptomic analysis revealed distinctPi16+Il33+Ly6c1+andCol15a1+Il33-Ly6c1-pancreatic fibroblast populations; using computational inference and experimental lineage-tracing models, we found thatPi16+fibroblasts represent an interstitial fibroblast population with

progenitor potential that can differentiate intoCol15a1+intraparenchymal fibroblasts. In acute pancreatitis, while ILC2-mediated regulation of steady-state fibroblast abundance augments tissue damage, activated ILC2s also promote early proliferation ofPi16+fibroblasts that replenish the depletedCol15a1+fibroblast population. This ILC2-Pi16+fibroblast axis is dysregulated in pancreatic cancer, resulting in the expansion of a peritumour fibro-immune niche during early tumour development that serves as a source of cancer-associated fibroblasts.

Conclusion: Pancreatic ILC2 inhabit an interstitial fibroblast progenitor niche and enforce homeostatic circuits that regulate steady-state, inflammatory, and cancer associated fibroblast densities.

Sawa, Shinichiro: Kyushu University

A hierarchical Rorc(γt) cis-regulatory cascade orchestrates differentiation of RORγt^{+} innate immune cells

Beyond its well-established role in type 3 immunity, RORγt+ innate immune cells are also essential for secondary lymphoid organ (SLO) formation and gut homeostasis. However, the transcriptional regulatory mechanisms governing RORγt expression in these cells, including ILC3s, LTi cells, and antigen-presenting cells (APCs), remain largely unknown. Here, we identify two key cis-regulatory elements within conserved non-coding sequences (CNS) 9 and 11 in the Rorc locus, which are sequentially utilized during their differentiation. Initially, Runx-binding sites in CNS11 establish chromatin accessibility as early as the HSC stage. Notably, disruption of this chromatin priming prevents subsequent transcriptional activation, thereby abolishing the initial induction of RORγt in these cells. At later stages, CNS9 plays a critical role, particularly in the development of RORγt^{+} APCs, contributing to colonic pTreg induction. This hierarchical transcriptional regulation is essential for SLO formation, postnatal type 3 immunity, and restraining excessive intestinal type 2 immune responses through pTreg induction.

ILC6 2026 Abstracts:

Poster Pitches- Alphabetical by Submitter

Chopra, Maggie University of British Columbia

- *Activated unconventional T cells masquerade as innate lymphoid cells in the inflamed lungs*

The pulmonary immune system is at constant interface with the environment, requiring rapid antimicrobial defenses without triggering harmful inflammation that disrupts alveolar gas exchange. Innate lymphoid cells (ILCs) are central to this balance. While pulmonary group 3 ILCs (ILC3) play both protective and pathogenic roles, their origins during inflammation remain unclear due to their scarcity in steady-state mouse lungs. We investigated these origins in the inflamed mouse lung by profiling non-productive T cell receptor (TCR) rearrangements found in ILCs as endogenous lineage barcodes. Our analysis revealed distinct patterns of TCRγ rearrangements in lung IL-18Rα+ ILC precursors, ILC2s, and ILC3s, indicating that these subsets are unlikely to share a common ancestor. Unexpectedly, however, the ILC3 repertoire showed a remarkable degree of overlap with unconventional double-negative αβ T cells. Using spectral flow cytometry, multiplexed scRNA-seq and TCR V(D)J profiling, we demonstrate that the pulmonary ILC3 population is highly susceptible to contamination by activated iNKT and MAIT cells. These unconventional αβ T cells downregulate lineage markers and adopt a transcriptional program convergent with ILC3. These findings reveal that the composition of the adult lung ILC3 compartment under acute inflammation is more complex than previously appreciated, challenging our current definitions of innate lymphocytes in inflamed tissues.

Coman, Diana: King's College London

- *ILC3s mediate intestinal immune-epithelial interactions via TGF-β1 activation*

Group 3 Innate Lymphoid Cells (ILC3s) are tissue-resident cells which contribute to intestinal homeostasis, in part through induction of tolerogenic T cell responses and maintenance of the intestinal epithelial barrier. However, during Inflammatory Bowel Disease (IBD), a multifactorial inflammatory disease of humans characterised by widespread immunological perturbations, ILC3 distributions and functionalities are significantly altered, potentially contributing to disease. Our objective was to uncover the mechanistic basis of ILC3s interactions with the intestinal epithelium and adaptive immune cells in health and in IBD.

We used a combination of murine and human experimental approaches, including co-cultures of ILC, T cells and intestinal organoids, and extensive analysis of intestinal ILC and epithelial datasets.

Here we show that ILC3s modulate intestinal epithelial and immune interactions through Transforming Growth Factor-Beta 1 (TGF-β1). ILC3s synthesise latent TGF-β1, and activate it through mechanical and proteolytic pathways, aiding induction of FoxP3+ regulatory T cells and promoting a regenerative transcriptional signature in intestinal epithelial cells. The impact of ILC3-derived TGF-β1 is conserved between mouse and humans, but the TGF-β1 activators expressed by ILC3 differ between the species. Importantly, ILC3s from inflamed colonic regions of human IBD samples maintain their expression of TGFB1 and its activators, suggesting that despite alterations to ILC3 population distributions during IBD, this pathway remains intact.

Intestinal ILC3s promote TGF-β1 signalling in epithelial cells and induce FoxP3+ regulatory T cells differentiation via TGF-β1, highlighting the significance of ILC3s in tissue remodelling and gut immune regulation. This pathway could potentially be targeted to promote a return to intestinal homeostasis in IBD.

Griffiths, Emma: MRC Laboratory of Molecular Biology

- *Zfp871: a new regulator of type-2 immunity*

Type-2 immunity is important for the clearance of parasitic helminth worms from mucosal surfaces. However, when dysregulated it can cause harmful pathologies such as asthma and allergies. Type-2 innate lymphoid cells (ILC2s) and CD4+ T cells coordinate this immune response through production of cytokines such as interleukin-4 (IL-4), IL-5, IL-9, and IL-13. To identify regulators of IL-13 production in ILC2s CRISPR screens were performed using lymphocyte progenitors from IL-13 reporter mice. Zfp871 was identified as a novel positive regulator of IL-13 production.

We confirmed the role of Zfp871 in type-2 immunity by analysing mice in which we conditionally deleted Zfp871. Initially, we deleted Zfp871 in all lymphocytes using Il7r-Cre (Il7rCreZfp871f/f, hereinZfp871IL7RKO). These mice and controls were treated intranasally with IL-33 which promotes pulmonary inflammation characterised by ILC2 proliferation and type-2 cytokine production. Although the numbers of lymphocytes were not altered, we observed fewer IL-13-producing ILC2s and a reduction in IL-5 and IL-13 producing T cells in the lungs. Next, we assessed the impact of lymphocyte-specific Zfp871-deficiency on immune responses to allergen extracts fromAlternaria alternata, a fungus which contributes to asthma development and exacerbations. Acute intranasal administration resulted in fewer IL-13-producing ILC2s and T cells. Furthermore, a papain/2W1S rechallenge model highlighted a striking failure in the outgrowth of 2W1S peptide-positive T cells, which were almost totally absent in the lungs ofZfp871IL7RKOmice. Together, these data demonstrate that Zfp871 promotes both acute IL-13-associated, innate type-2 immune responses and antigen-specific Th2 immunity in the lung. Investigations are underway to dissect the relative importance of Zfp871 in ILC2s and T cells using Boolean-ILC2-Cre (BIC) andCd4Cremouse lines. Ongoing

studies to determine the underlying mechanisms by which ZFP871 regulates type- immunity will be discussed.

Huang, Qiutong: University of Calgary

- *ILC2 promotes breast cancer development and progression through IL-13-dependent reprograming of immunosuppressive tumour-associated macrophages.*

Introduction: Innate lymphoid cells (ILCs) are immune cells that respond rapidly to microenvironmental signals to initiate immune responses. We and others found that type 2 ILCs (ILC2s) drive type 2 immune response and can contribute to tumor development and progression, although both pro- and anti-tumour effects have been reported. ILC2 accumulate in breast tumours but their role in tumor progression has not been examined.

Methodology: To investigate the role of ILC2s in breast cancer, we used spontaneous (PyMT) and orthotopically injected (AT3) breast cancer mouse models in ILC2-deficient RORafl/flIL-7RCre mice and ILC2-sufficient littermate controls to examine changes in breast tumor development in the absence of ILC2s. scCITEseq was performed on healthy mammary fat pad and PyMT tumours from ILC2-sufficient and deficient mice to identify the changes to the tumour microenvironment during breast cancer development and in the absence of ILC2. Macrophage polarisation was assessed by co-culturing IL-13-deficient (IL-13eGFP/eGFP) or sufficient (IL-13eGFP/+) ILC2 with bone marrow-derived macrophages (BMDM) for 72h and macrophage phenotype was characterised by flow cytometry.

Results: ILC2-deficient mice exhibited delayed tumor onset and reduced growth compared to littermate controls in both PyMT and AT3 breast cancer models. scCITE-seq analysis identified a cluster of immunosuppressive tumour associated macrophages (TAMs) in PyMT tumors that was lost in the absence of ILC2, with a similar trend observed in AT3 tumors analysed ex vivo. CellChat analysis predicted IL-13 signalling from ILC2 to TAMs. Consistently, IL-13 deficiency in ILC2 coculture or conditioned media significantly reduced expression of immunosuppressive markers (Arg1 and CD206) in BMDMs.

Conclusions: These findings suggest that ILC2 within the breast tumour microenvironment act in a pro-tumor manner to promote onset of breast cancer development and progression of tumor growth. This occurs in part through the production of IL-13 to induce immunosuppressive TAM in the tumour microenvironment.

Kaur, Simran Preet: All India Institute of Medical Sciences

- *Lactobacillus acidophilus (LA) supplementation mitigates inflammatory bone loss by modulating the "ILC3-ILCreg" cell axis*

Introduction: Immunoporotic potential of the “Treg-Th17” cell-axis has been extensively studied in post-menopausal osteoporosis (PMO). However, the role of Innate Lymphoid Cells (ILCs), which represent the innate counterpart of helper-T cell subsets, is yet to be explored in

PMO. Additionally, while our laboratory has demonstrated immunomodulatory potential of probiotics in regulating the "Treg-Th17" cell-axis, their effects on the ILCs remain unexplored. Therefore, this study aims to examine the potential of a probiotic, Lactobacillus acidophilus (LA) in modulating the "ILC3-ILCreg" cell axis in murine model of Osteoporosis.

Materials & Methods: Building on the novel concept of "Immunoporosis", we investigated the role of the "ILC3-ILCreg" cell axis in PMO and evaluated the Immunoporotic potential of LA supplementation in restoring the same. Female (C57BL/6J) mice were ovariectomized (OVX) and divided into Sham, OVX and OVX+LA groups (n=6/group). 45 days post-surgery, mice were sacrificed, allowing us to evaluate a range of osteoimmune parameters (ILC3-ILCreg, Treg-Th17) in the bone-marrow and mucosal tissues, along with serum cytokines (IL-17, IL-10, IL-22, etc.), and micro-CT analysis.

Results: Interestingly, our findings reveal that PMO conditions significantly disrupted gut integrity, and enhanced RORγt+CCR6+ ILC3s which was accompanied by a concomitant reduction in the anti-inflammatory ILC populations i.e. RORγt+CCR6- ILC3s and GATA3+ IL10+ ILCregs. Concomitantly, serum cytokine balance was tipped towards the inflammatory phenotype suggesting a crucial role of ILC3-ILCreg cell axis in inflammatory bone loss in OVX mice. Notably, the administration of LA ameliorates these effects by restoring the osteoimmune profiles (in vivo and in vitro).

Conclusions: Our findings for the first time provide compelling evidence for the osteoprotective potential of LA by modulating the ILC3-ILCreg cell-axis in the murine model of PMO, thus paving the way for clinical applications.

Kobayashi, Tetsuro: RIKEN

- *Mechanical stress promotes skin barrier resilience via P2RX1–AREG signaling in ILC1*
  ILC Tissue-Residency, Niches, and Tissue Remodeling

Skin-resident immune cells are essential for tissue homeostasis, yet how they respond to low-level mechanical stress such as scratching and grooming remains unclear. Although excessive scratching in atopic dermatitis can drive inflammation, routine scratching and grooming are thought to help clear foreign material from the skin surface without overt inflammation. In this study, we reveal a previously unrecognized mechanism in which skin-resident ILC1s act as highly sensitive sensors of mild mechanical stress by detecting extracellular ATP and promoting epithelial turnover through amphiregulin (AREG) production. Mechanical stress that removes only superficial layers of the stratum corneum induced epidermal hyperplasia in WT and Rag2−/− mice, whereas this response was markedly blunted in Rag2−/−Il2rg−/− mice, indicating a key role of ILCs. Analysis of single-cell RNA-seq of skin ILCs revealed an underappreciated ILC1 population in the skin. In IL-15–deficient settings in which skin ILC1s are absent, the stress response was profoundly reduced, suggesting that ILC1s are required. We

further found that mild mechanical stress triggers extracellular ATP release from keratinocytes. This purinergic signal induces AREG production by ILC1s via the ATP receptor P2RX1, promoting epithelial turnover by accelerating basal-layer proliferation and differentiation and by facilitating stratum corneum shedding through kallikrein-related peptidases. Disrupting either ATP signaling (P2rx1−/− mice) or ILC1-derived AREG production (Ncr1-iCre Aregfl/fl mice) impaired epithelial renewal after mechanical stress and delayed the clearance of bacteria and allergenic substances from the skin surface. Consequently, mechanical stress–mediated ILC1 activation protected against atopic-like skin inflammation. Collectively, our findings reveal that mechanical stress, often viewed as a pro-inflammatory cue, instead promotes skin resilience through an ATP–ILC1–AREG pathway. Our study underscores that, under homeostatic conditions, transient low-intensity scratching can be physiologically beneficial by engaging an innate immune–sensing system to help clear foreign materials from the skin surface.

Mashhouri, Siavash: University of Toronto

- *Engineering Chimeric Antigen Receptor-ILC2s for Cellular Therapy*

IL-10-producing group 2 innate lymphoid cells (ILC210) have emerged as key regulators of inflammation. We previously reported that human ILC210inhibit T cell-driven pathology in humanized models of graft-versus-host disease (GVHD) model and islet transplantation. In both settings, cell therapy with ILC2s suppressed T cell proliferation and cytokine production, and reduced tissue injury. While these studies provide proof-of-principal for ILC210cell therapy applications, their therapeutic potential is constrained by their lack of antigen specificity and dependence on local microenvironmental factors for activation. To improve cell therapeutic efficacy and control ILC2 activation, we generated and functionally validated chimeric antigen receptor (CAR)-engineered ILC210. We report that CAR-engineered human ILC210can restrain T cell-dependent pathology in a xenogeneic GVHD model more efficiently than unmodified ILC210. Using CITE-seq and flow cytometry, we identified stimulatory domains of interest, including CD28, GITR and ICOS, and evaluated their potential when incorporated into CARs to enhance ILC210function. We compared antigen-specific activation, proliferation, and cytokine production of different CAR constructsin vitro, and noted varying effects on ILC210cytokine production. Next, we assessed their ability to enhance ILC210activityin vivo. Here, CAR-ILC210incorporating CD28 as a co-stimulatory domain enhanced cell therapy efficacy and improved overall survival in the xenoGVHD model compared to non-engineered ILC210. Furthermore, trafficking studies showed that CAR-ILC210persisted longer than non-engineered ILC210following adoptive transfer. Ongoing studies, including bulk RNA-seq and co-culture assays, are exploring mechanisms involved in the enhancedin vivofunction of CAR-ILC210and how CD28 impacts human ILC210function. Together, these findings establish the feasibility of engineering human ILCs for immunotherapy and provide proof-of-principle that CAR-

ILC210represent an adaptable platform for targeted therapies and provide new insights into pathways that modulate human ILC210activity.

Riesebosch, Samra: Amsterdam UMC

- *Human type 3 innate lymphoid cell-derived Amphiregulin promotes pancreatic ductal adenocarcinoma progression*

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with a poor prognosis, characterized by a profoundly fibrotic and immunosuppressive tumor microenvironment (TME) that supports tumor progression. Here we identify the epidermal growth factor (EGF) family member, amphiregulin (AREG), as a central mediator linking tumor growth, fibrosis, and immune suppression in PDAC. Using flow cytometry and single-cell transcriptomic approaches, we identified a subset of type 3 innate lymphoid cells (ILC3s) as the predominant source of AREG in lymphocytes derived from freshly isolated human PDAC tissues. AREG expression was specific to tumor-infiltrating ILC3s, as matched duodenal ILC3s virtually lacked AREG expression. While ILC3s accumulated in PDAC tumors at frequencies comparable to duodenal tissues, they were largely absent from unaffected pancreas tissues.

Functionally, primary isolated ILC3s, isolated from blood, duodenum, or naïve fetal pancreas, as well as clonally expanded tonsillar ILC3s, induced AREG production following stimulation with pro-inflammatory cytokines, but only in conjunction with tumor growth factor beta (TGF-β), a cytokine abundantly present in the PDAC TME. Using patient-derived PDAC tumoroids, we demonstrate that amphiregulin does not enhance proliferation, but induces PD-L1 expression in tumoroids, as revealed by fluorescent reporter tumoroids in which a red fluorescent tag was fused to the terminal exon of the PD-L1 encoding gene.

Together, these findings demonstrate that the PDAC TME is sufficient to rapidly reprogram infiltrating ILC3s towards a tumor promoting phenotype by inducing high Amphiregulin levels, and thereby directly coupling immune remodeling and immune evasion.

Roger, Nolwenn: Inserm U1231

- *ILC3 and T lymphocytes: exploring immune interactions for the development of innovative combination therapies in cancer*

Colorectal cancer remains a major therapeutic challenge, partly due to the strong immunosuppressive environment associated with tumor progression. The chemotherapy 5-fluorouracil (5-FU) is a standard treatment of colorectal cancer. Its impact on the tumor immune landscape extends beyond cytotoxicity and remains insufficiently understood, particularly regarding innate lymphoid cells (ILC) and T-cell regulation.

We investigated how 5-FU shapes innate and adaptive immunity, focusing on group 3 innate lymphoid cells (ILC3). In the CRC murine model CT26, 5-FU not only depletes myeloid-derived suppressor cells but also induces a notable increase in intratumor CCR6$^+$ ILC3. This accumulation coincides with an increased proportion of regulatory T cells (Tregs), indicating a profound reorganization of the CD4 T-cell compartment following chemotherapy. The correlation between ILC3 accumulation and Treg increased frequency suggests a functional interaction, where ILC3 may support the recruitment or expansion of Tregs, potentially limiting antitumor T-cell responses.

To investigate the functional contribution of ILC3 to 5-FU–induced immune remodeling, we performed a depletion of RORγt$^+$ cells during treatment that resulted in enhanced tumor growth compared to 5-FU alone and was accompanied by significant alteration in both CD4 and CD8 T cells compartments. Although ILC3 depletion reduced Treg population, it also impaired effector responses, as evidenced by a decreased frequency of IFN-γ- and granzyme B-expressing CD8 T cells.

Collectively, these findings indicate that ILC3 are critical modulators of T-cell responses during chemotherapy. 5-FU-mediated tumor control is associated with ILC3 presence and coordinated regulation of regulatory and cytotoxic T-cell populations. Targeting the ILC3–Treg axis may represent a promising strategy to enhance chemotherapy-induced antitumor immunity in colorectal cancer.

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- *Dupilumab treatment in severe asthma is associated with enrichment of transcriptionally modified circulating ILC2*

Asthma affects 9% of Europeans, and 4% of these patients suffer from severe disease that cannot be controlled with inhaled corticosteroids. Severe asthma is marked by persistent inflammation, tissue damage, and frequent exacerbations, leading to major morbidity, increased mortality, and healthcare costs. Although current biologics suppress type 2 immune responses, many patients fail to regain lung function or achieve long-term disease control. We used high resolution immunophenotyping and single cell V(D)J profiling to examine how anti IL 4Rα (dupilumab) therapy alters type 2 lymphocytes in patients with severe asthma. ILC2 were increased in the circulation of patients 1 year after dupilumab treatment compared to baseline, and scRNA-seq of post-treatment ILC2 showed a reduction in markers of proliferation (GATA3, FKBP5) and in molecules involved in homing to lymphoid structures (SELL, CD7). In addition, we detected increased expression of HPGDS transcript in dupilumab-treated ILC2, suggesting that circulating ILC2 are primed to produce prostaglandins after biologics treatment. After PMA and ionomycin stimulation, ILC2 from dupilumab-treated patients produced as much IL-5 and IL-13 as pre-treatment ILC2, suggesting that despite the transcriptional changes induced by IL-4 receptor blockade, ILC2 are still functional type 2 cytokine producers. Given the

enrichment ILC2 in the circulation during dupilumab treatment, this raises concerns about how ILC2 could contribute to asthma exacerbation upon treatment cessation or tapering. Our results contribute to an increased understanding of the downstream consequences of anti-type 2 biologics treatment.

Tai, Siu Ling: University of Toronto

- *pH-dependent regulation of postnatal ILC3*

Group 3 innate lymphoid cells (ILC3) are the dominant subgroup of gut-resident ILC, with increasing density towards the distal regions of the gut in correlation with microbial diversity post weaning. Early in life, ILC3 regulate lymph node organogenesis, while maintaining epithelial barrier integrity and defense against enteric pathogens in adults. The broad functional diversity of ILC3 highlights an underappreciated heterogeneity that may reach beyond the currently accepted classification of ILC3 subsets. Intriguingly, there have been multiple reports of ILC3-specific expression of KCNK1, a potassium channel, suggestive of additional unique ILC3 subsets with specialized functions.

Using a KCNK1 reporter mouse strain, we identified that KCNK1 is uniquely expressed on gut-resident NCR- ILC3 which accumulate within isolated lymphoid follicles. Notably, KCNK1+ ILC3 are upregulated at time of weaning, suggesting that KCNK1+ ILC3 accumulation is driven by luminal factors. To determine the developmental origin of KCNK1+ ILC3, we performed adoptive transfers of purified KCNK1- and KCNK1+ ILC3 into Rag2-/-Il2rg-/- mice and found that KCNK1+ and KCNK1- ILC3s constitute two distinct fates at homeostasis, while KCNK1- neonatal precursors give rise to KCNK1+ cells in adult mice. Single cell RNA sequencing revealed that KCNK1+ ILC3 possess a distinct metabolic profile and reduced migratory capacity compared to KCNK1- ILC3. Functionally, KCNK1+ ILC3 produced fewer cytokines, a phenotype that requires microbial signals.

Inflammatory processes cause extracellular acidification in the local environment. KCNK1 undergoes a conformational closure at acidic pH of 5.5, inspiring us to investigate the effect of pH changes on KCNK1+ ILC3 function. Indeed, KCNK1+ ILC3 exhibited altered migratory capacity and heightened effector responses to decreases in extracellular pH, which were paralleled by changes in chromatin structure.

These findings demonstrate that KCNK1+ ILC3, which arise postnatally, constitute a distinct subset that is uniquely regulated by environmental pH.

Tatematsu, Megumi: Akita University Graduate School of Medicine

- *CNOT3 preserves ILC2 identity by post-transcriptionally limiting T-bet and RORγt*

Innate lymphoid cells (ILCs) comprise T-bet–dependent NK and ILC1 cells, GATA-3–dependent ILC2 cells, and RORγt–dependent ILC3 cells. However, the post-transcriptional mechanisms governing their functional differentiation and lineage stability remain poorly understood. The CCR4–NOT complex is a key regulator of mRNA turnover, primarily through its role in deadenylation. To investigate the contribution of mRNA decay to ILC biology, we conditionally ablated Cnot3, an essential component of the CCR4–NOT complex. CNOT3 deficiency in ILC2 cells resulted in aberrant expression of T-bet and RORγt, accompanied by induction of type 1 and type 3 gene signatures. Mechanistically, CNOT3 promoted the degradation of Tbx21 and Rorc transcripts by targeting their 3′ untranslated regions via interactions with Roquin and ZFP36L1, respectively. Increased T-bet expression in CNOT3-deficient ILC2 cells led to reduced GATA-3 levels, thereby compromising type 2 immune responses in models of allergic airway inflammation and helminth infection. Collectively, these findings demonstrate that CNOT3 preserves ILC2 lineage identity by constraining alternative type 1 and type 3 transcriptional programs at the post-transcriptional level.

Winkler, Etienne: Karolinska Institutet

- *Atopic dermatitis predisposes mice to ILC2-mediated allergic lung inflammation*

Atopic dermatitis (AD) is an allergic disease associated with the later development of allergic asthma, yet the mechanisms underlying a potential skin–lung axis remains elusive. Here, we show that AD-like skin inflammation induced by topical treatment with the Vitamin D agonist calcipotriol (MC903) triggers systemic immune cell alterations, including changes in lung group 2 innate lymphoid cells (ILC2s). Upon AD-like skin inflammation, we observed an accumulation of proliferating ST2hi ILC2s in the lung. Single cell RNA sequencing (scRNA-seq) revealed the emergence of a transcriptionally distinct “primed” ILC2 state in calcipotriol treated mice, characterized by a signature of cell cycle progression and increased expression of key cytokines. Functionally, mice with AD like skin inflammation mounted stronger immune responses upon concomitant intra-nasal exposure to papain, with increased ILC2 expansion accompanied by increased eosinophil recruitment. Furthermore, we show that this enhanced response is independent of T cells. This primed state was transient, yet concomitant allergen exposure during skin inflammation imprinted long lasting enhanced lung ILC2 responsiveness. Notably, neonatal AD like skin inflammation predisposed adult mice to heightened ILC2 activation and eosinophilia upon airway IL-33 challenge. Together, these findings identify remote priming of lung-resident ILC2s during AD-like skin inflammation and uncover a mechanistic connection for the progression from AD to allergic diseases in the lung.